Evidence in Support of Hypoxia but Against High Potassium and Hyperosmolarity as Possible Mediators of Sustained Vasodilation in Rabbit Cardiac and Skeletal Muscle

By Miklos Gellai and Reed Detar

ABSTRACT
The vasoactive properties of hypoxia, elevated extracellular potassium concentration ([K\textsubscript{o}]), and hyperosmolarity were studied in helical strip preparations of small coronary and deep femoral arteries (260-700 \textmu m, o.d.) equilibrated in a physiological salt solution with an oxygen tension (Po\textsubscript{O}) of 100 mm Hg, [K\textsubscript{o]} of 3.18 mM, and an osmolarity of 304 milliosmoles/liter. Increasing [K\textsubscript{o}] (2-6 mM) or osmolarity (30-50 milliosmoles/liter) produced relaxation of resting tension in 50% of the coronary strips but had no effect on resting tension in deep femoral strips. Sustained increments in [K\textsubscript{o}] or osmolarity produced concentration-dependent, transient relaxation of agonist-induced contractile tension in both coronary and deep femoral arterial strips: a potassium increment of 4 mM produced 40% relaxation with 100% recovery within 5-6 minutes, an osmotic increment of 30 milliosmoles/liter caused 20-40% relaxation with 100% recovery within 15-60 minutes, and simultaneous potassium (4 mM) and osmotic (30 milliosmoles/liter) increments produced 85-95% relaxation with 100% recovery within 10-15 minutes. Decreasing Po\textsubscript{O} from 100 mm Hg to 10 mm Hg resulted in a sustained 35-40% fall in agonist-induced contractile tension. Although a nonspecific additive interaction was observed during a simultaneous change to high [K\textsubscript{o}], hyperosmolarity, and hypoxia, for any given level of vascular tone hypoxia had little or no effect on the degree or the duration of the tension relaxation-recovery sequence produced by elevated [K\textsubscript{o}], hyperosmolarity, or both. Therefore, it is proposed that hypoxia is the only one of these three factors that, by a direct interaction with vascular smooth muscle cells, can contribute to sustained vasodilation of small arteries in rabbit cardiac or skeletal muscle.

KEY WORDS
- acetylcholine
- local control of blood flow
- coronary artery
- physiological salt solution
- osmolarity
- deep femoral artery
- oxygen
- epinephrine

Much research has been directed toward identifying those factors local to the resistance vessels that account for the vasodilation observed in skeletal muscle during active hyperemia (1, 2). Of the several factors that have been considered as possible candidates for this role, the three which have recently received the most attention are hypoxia, elevated extracellular potassium concentration ([K\textsubscript{o}]), and hyperosmolarity. Little is known about the direct interaction between these factors and the vascular smooth muscle cells. Since the isolated vessel technique represents a vitally important tool for investigating this interaction, we initiated the present study on small arteries from both cardiac and skeletal muscle. From the present study, several important points have emerged which further our understanding of both the direct interactions between these three chemical factors and the vascular smooth muscle cells and the possible relative roles of these interactions in the onset and the maintenance of locally induced vasodilation in either cardiac or skeletal muscle.

Methods
Forty-three New Zealand white rabbits weighing 2-3 kg were used in this study. The rabbits were killed by a posterior cervical blow, and helical strips were cut from vessel segments (260-700 \textmu m, o.d.) taken from coronary arteries removed from within the free wall of the left ventricle or from deep femoral arteries removed from within the adductor magnus muscle. All vessel strips were cut at approximately a 65-75° angle with respect to the longitudinal axes of the isolated vessel segments by making the width of each strip approximately equal to the diameter of the vessel segment from which it was cut. Thicknesses of the strips ranged from 20 \textmu m to 60 \textmu m for...
coronary arteries and from 40 μm to 80 μm for deep femoral arteries. These dimensions were measured using a reticle in a Bausch & Lomb Stereo 70 Zoom dissecting microscope. The length of the strips at zero resting tension between the tie points used for suspending them in the tissue bath was between 6 mm and 9 mm.

The strips were suspended in a physiological salt solution with the following millimolar composition: NaCl 119, KCl 2, KH₂PO₄ 1.18, MgSO₄ 1.17, NaHCO₃ 14.9, CaCl₂ 1.6, dextrose 5.5, sucrose 20, CaNa₂ ethylenediaminetetraacetate (EDTA) 0.026. Temperature and pH in the bath were held constant at 37 °C and 7.25, respectively. A normoxic gaseous environment, i.e., an oxygen pressure (PO₂) of approximately 100 mm Hg, was maintained by bubbling a 5% CO₂-15% O₂-80% N₂ mixture from the bottom of the tissue bath. PO₂ was measured with a Beckman macroelectrode placed in the bath in proximity to the tissues. Isometric tension of the strips was measured with a Grass FT03 linear force transducers. PO₂ and mechanical tension were recorded continuously on a Grass model 7 polygraph.

Two or three strips from one site only (cardiac or skeletal muscle) for any given experiment were equilibrated in the tissue bath at a PO₂ of 100 mm Hg for approximately 90 minutes prior to testing. Initial resting tension was adjusted to between 50 mg and 150 mg according to the size of each strip. This tension produced a strain of 20% of the resting length at zero tension and was optimum for agonist-induced responsiveness of these strips (unpublished observations). Twenty-one of 45 coronary arterial strips demonstrated a spontaneous increase in resting tension during the equilibration period (the initial 90-minute period) or following the first agonist-induced contractile response (after 90 minutes). This spontaneous tone could be relaxed by vasodilators such as nitroglycerin, adenosine, elevated [K+]p, or hyperosmolarity. The level of spontaneous tone observed was usually < 20-25% of the maximum tension that could be produced by an agonist and was present for varying periods of time, often intermittently.

At 90 minutes, a contractile response was produced with an intermediate concentration of agonist. After 5-15 minutes, contractile tension was relaxed by rinsing the tissues three times over 10-15 minutes. This contractile-relaxation cycle was repeated until steady-state responsiveness was attained and testing could be undertaken. The agonist most often used to produce active tension in coronary arterial strips was acetylcholine; in deep femoral arterial strips it was epinephrine. Norepinephrine was also tested as an agonist for the deep femoral arterial strips and produced results that were entirely similar to those produced using epinephrine. These agents were used because they produced consistent, reproducible responsiveness of strips from one experiment to another. Plasma obtained from fresh heparinized rabbit blood and histamine were occasionally used as agonists for both types of vessel strips. The concentrations of agonist used to produce active tension for testing the effects of added potassium, hyperosmolarity, or hypoxia were chosen so that tension development was greater than 50% but less than 90% of the maximum tension that could be produced by the respective agonist (based on dose-response relationships obtained at the beginning or the end of each experiment).

Potassium concentration in the bath ([K+]p) was increased by directly injecting small volumes (0.25 ml or less) of a 2M KCl solution. At times potassium concentration was maintained high by rinsing the strips with a physiological salt solution with the KCl concentration adjusted to a high level. Osmolarity was increased 30 milliosmoles/liter in the tissue bath by directly administering 1.5 ml of a 1M sucrose solution. Osmotically equivalent injections of NaCl were also made using 0.38 ml of a 2M NaCl solution. Osmolarity was maintained at an elevated level by using a physiological salt solution containing a high sucrose concentration. The osmotic increments produced by added KCl and NaCl are described using the assumption that the osmotic coefficient is unity. The PO₂ in the tissue bath was reduced to approximately 10 mm Hg by bubbling the perfusion fluid with a 5% CO₂-1.5% O₂-93.5% N₂ gas mixture. This low PO₂ level, which represents a severe hypoxic stress, was chosen for the present study to ensure that any effects on vascular tone that resulted from the interaction of hypoxia, elevated [K+]p, and hyperosmolarity would be revealed. This choice was prudent since our findings led to the conclusion that, except for a nonspecific effect, hypoxia did not influence the effects of elevated [K+]p or hyperosmolarity on vascular tone. Whenever PO₂ was changed in the tissue bath, the new level was maintained for at least 5 minutes before testing was initiated.

Acetylcholine, epinephrine (Adenalin chloride solution, Parke-Davis), norepinephrine (Levophed, Winthrop Laboratories), and histamine dihydrochloride were made up into stock solutions which were kept on ice throughout each experiment. These agonists were each administered directly into the 50 ml tissue bath at volumes between 0.1 ml and 0.5 ml. The concentration of each of these agents is expressed in terms of final bath concentration.

**Results**

**Effects of High Extracellular Potassium Concentration**

When the background [K+]p was 5.88 mM, added potassium (2-6 mm) produced no apparent change in either spontaneous or agonist-induced contractile tension. Hence, for all of the experiments reported in the present study, the [K+]p in the physiological salt solution used for equilibrating both coronary and deep femoral arterial strips was 3.18 mM.

Increasing [K+]p by 1 mM or more above the equilibrating level, 3.18 mM, produced abrupt relaxation of resting tension in the coronary arterial strips that demonstrated spontaneous tone (point a, Fig. 1A). The potassium-induced relaxation of this low level of tone lasted at least 15-30 minutes. But, if an agonist-induced contracture was produced during this period of depression, after the agonist had been washed from the bath with [K+]p maintained at the elevated level, resting tone then quickly stabilized at a level above that which was present at 3.18 mM [K+]p (points b and c, Fig. 1A).
Effect of increasing $[K]_o$ from 3.18 mM to 7.18 mM on resting tension and on contractile responses to acetylcholine of two different helical coronary arterial strips. A broken line represents the absolute resting tension level that was present prior to increasing $[K]_o$.

A: Sustained increase in $[K]_o$ produced by adding KCl caused a fall in resting tension and a potentiation of the next acetylcholine-induced contractile response, i.e., net response was 250 mg compared with the control response of 140 mg. Following this response, resting tension remained above control, and, although the subsequent acetylcholine-induced contraction was slightly depressed (net response of 120 mg), the peak contractile tension that developed during this response was above that obtained at 3.18 mM $[K]_o$.

B: Increasing $[K]_o$ did not produce a fall in resting tension of this coronary strip. Contractile responses to acetylcholine were enhanced at the higher $[K]_o$, so that peak tension was above that obtained at 3.18 mM $[K]_o$. A broken line represents point of rinsing the bath; numbers in parentheses indicate the net amplitude (mg) of respective contractile responses. In both A and B, traces are continuous with respect to time from top to bottom.

Figure 1A and B demonstrates that the peak active tension (spontaneous plus agonist-induced tension) associated with a given agonist concentration was augmented at higher $[K]_o$. It is also apparent from Figure 1 that the net amplitude of the contractions produced by the agonist depended on whether resting tension was affected by potassium; contractions were augmented at the higher $[K]_o$ if resting tension was below control or unchanged but were depressed if resting tension was increased.

Potassium added to the tissue bath during agonist-induced contraction produced abrupt relaxation of contractile tension in both coronary and deep femoral arterial strips. Table 1 indicates that an increase in $[K]_o$ of 4 mM produced similar relaxation (about 40%) in coronary and deep femoral arterial strips. Equivalent molar increments of extracellular sodium concentration ($[Na]_o$) (injected as NaCl solution) produced no change in contractile tension. For any given strip, the degree of relaxation produced by increasing $[K]_o$ was inversely related to the size of the agonist-induced test response (Fig. 2). Hence, a reliable dose-response relationship was obtained for each strip by testing the effects of three different increments (2, 4, and 6 mM $[K]_o$) on similar-sized agonist-induced test responses (Table 2). When testing was carried out in this fashion, coronary vessel strips appeared to be only slightly more sensitive to the relaxing effects of potassium than were strips from deep femoral vessels (Table 2).

Figure 3 and Tables 1 and 2 show that the relaxation produced by potassium was transient. Tension was recovered back to the control level approximately 5 minutes after the onset of relaxation for both coronary and skeletal muscle vessels. Although the time for recovery was also indirectly related to the size of the test contractions (25-90% of maximum) as was the degree of relaxation produced by increasing $[K]_o$, these variations in recovery time were less than ±2 minutes. However, if agonist-induced tension was < 20-25% of maximum, the duration of this potassium-induced relaxation was prolonged and similar to that observed for spontaneous tone. Following recovery, tension often reached levels above control. In 32 of the 49 strips included in Table 1, a steady-state level 10-40% above the prerelaxation peak tension level was attained.

Effects of Hyperosmolarity

Increasing osmolarity in the tissue bath medium produced a fall in resting tension in the same strips in which resting tension was relaxed by potassium, i.e., in those coronary arterial strips demonstrating

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TABLE 1

<table>
<thead>
<tr>
<th>Test condition</th>
<th>Coronary arterial strips</th>
<th>Deep femoral arterial strips</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relaxation (%)</td>
<td>Recovery time (minutes)</td>
</tr>
<tr>
<td>High [K], (7.18 mM)</td>
<td>42 ± 4.9</td>
<td>5.5 ± 0.3</td>
</tr>
<tr>
<td>Hyperosmolarity (334 milliosmoles/liter)</td>
<td>35 ± 2.6</td>
<td>&gt;15</td>
</tr>
<tr>
<td>High [K], (7.18 mM) + hyperosmolarity (342 milliosmoles/liter)</td>
<td>96 ± 2.4</td>
<td>10.3 ± 0.43</td>
</tr>
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</table>

Contractile responses between 50% and 90% of maximum were produced by acetylcholine for coronary arterial strips or epinephrine for deep femoral arterial strips. Increments in [K], (4 mM) or osmolarity (30 milliosmoles/liter) were made during the steady-state contractile responses produced by these agonists (Figs. 2 and 4). The degree of relaxation is expressed as a percent ± se of the steady-state drug-induced tension present before each increment. Recovery time gives the time after onset of relaxation for 100% recovery of tension. Tension often reached a new steady state later in time at a level above control. N = number of arterial strips involved in each observation.

spontaneous active tension. Resting tension in the remaining 50% of the coronary arterial strips and in the deep femoral arterial strips was not affected by an osmotic increment. Hyperosmotic-induced depression of resting tension was not sustained, and tension was recovered to control levels between 15 and 60 minutes with or without intermittent agonist stimulation. Figure 4 illustrates an example of the effect of hyperosmolarity on resting tension in a coronary arterial strip.

During the 15-60-minute period after osmolarity had been increased, the peak active tension (spontaneous plus agonist-induced tension) associated with a given agonist concentration was depressed. Figure 4 shows a depression which lasted for approximately 30 minutes. This same effect was also observed in strips (including all deep femoral arterial strips) in which resting tension was unchanged by the osmotic increment. The actual net agonist-induced contractile response was augmented in the strips in which resting tension had fallen initially (Fig. 4) and was depressed in strips in which resting tone was unaffected by the hyperosmotic change (not shown). In all cases, agonist-induced contractile responses were back to control levels, i.e., back to prehyperosmotic levels, between 15 and 60 minutes.

Figure 5 illustrates the tension-relaxing effect produced by increasing osmolarity during agonist-induced contractile responses of coronary and deep femoral arterial strips. A 30-milliosmoles/liter increment in sucrose or NaCl produced a 20-40% fall in tension (Table 1). A 3% dilution of the salt concentrations in the bath produced by injecting 1.5 ml of distilled H₂O on top of an agonist-induced contraction, a procedure which simulated the salt dilution that occurred when 1.5 ml of sucrose solution was added to the bath, caused contractile tension to rise < 10%. As with potassium-induced relaxation, for any given strip the degree of relaxation produced by increasing osmolarity was inversely related to the size of the test contraction (Fig. 6).

Tension recovered slowly following hyperosmotic-induced relaxation. Tension usually recovered approximately 50% of the initial fall within 15
LOCAL CONTROL OF VASCULAR TONE

TABLE 2

Degree of Relaxation and Time for Recovery of Contractile Tension following Increments in [K⁺].

<table>
<thead>
<tr>
<th>Potassium increment</th>
<th>Coronary arterial strips</th>
<th>Deep femoral arterial strips</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Relaxation (%)</td>
<td>Recovery time (minutes)</td>
</tr>
<tr>
<td>2 mM</td>
<td>16 ± 2.8</td>
<td>6 ± 0.87</td>
</tr>
<tr>
<td>4 mM</td>
<td>42 ± 7.95</td>
<td>5.2 ± 0.39</td>
</tr>
<tr>
<td>6 mM</td>
<td>81 ± 11.42</td>
<td>6.4 ± 0.48</td>
</tr>
</tbody>
</table>

Data were obtained and are presented as described for Table 1 except that the effects of all three potassium increments (in random sequence) were tested in each strip on top of three consecutive contractile responses which varied < 5% in amplitude from one another and were 65-80% of maximum.

minutes, i.e., tension recovered to a level 10-20% below the prerelaxation control tension (Fig. 5). To follow recovery beyond the initial 15-minute period, the hyperosmotic condition was maintained while the agonist was rinsed from the tissue bath. Subsequently, the strips were restimulated with the same concentration of agonist. Normal responsiveness was usually observed with the first new stimulation (30 minutes) and was always seen with the second stimulation (60 minutes). In contrast to recovery following potassium-induced relaxation, there was no obvious correlation between the time of recovery following hyperosmotic-induced relaxation and the size of the contractions used for testing.

Four arterial strips (one coronary, three deep femoral) were tested using a 50-milliosmoles/liter increment (2.5 ml of 1M sucrose added to the tissue bath). Tension relaxation was approximately twice that produced by 30 milliosmoles/liter, and tension was recovered to a level 10-20% below control at 30 minutes.

COMBINED EFFECTS OF HIGH EXTRACELLULAR POTASSIUM CONCENTRATION AND HYPEROSMOLARITY

Table 1 indicates that simultaneous elevation of [K⁺] and osmolarity produced 85-95% relaxation of...
agonist-induced tone. This degree of relaxation was considered to represent an additive interaction, since it was greater than that produced by equivalent increments in [K]o or osmolarity administered separately. To us, the term additive interaction means simply that increasing the number of changes imposed in the local chemical environment produces increasing depression of vascular tone. Recovery of tension following relaxation produced by simultaneous exposure of the strips to hyperosmolarity (342 milliosmoles/liter) and elevated [K]o (7.18 mM) was fully established at 10-15 minutes. Figure 7 illustrates an example of an extreme hyperosmotic stress (50-milliosmoles/liter increment) which by itself produced relaxation followed by slow recovery (> 30 minutes). When this stress was applied in conjunction with a simultaneous elevation in [K]o , recovery of tension was complete at 22 minutes.

The effects of increasing osmolarity on resting tension or on agonist-induced contractile tension were the same whether the strips were equilibrated at 3.18 mM or 7.18 mM [K]o . Similarly, the effects on resting and agonist-induced tensions of increasing [K]o above 3.18 mM were the same in strips under steady-state conditions at 304 and 334 milliosmoles/liter. However, for a short period after increasing osmolarity, potassium-induced relaxation of agonist-induced contractile tension was augmented (Fig. 8A). Figure 8B demonstrates that this additive interaction between potassium and osmolarity could be reproduced by simply reducing the concentration of agonist to produce a reduced contractile response (center response) equivalent to that obtained with the original agonist concentration during the transient depression produced by hyperosmolarity (center response in Fig. 8A).

EFFECTS OF HYPOXIA

Resting tension did not change during the 5-10 minutes after Po2 was lowered to a hypoxic level of
Nonspecific nature of the additive interaction between hyperosmolarity and potassium in relaxing contractile tension of a deep femoral arterial strip. A: Potassium-induced relaxation was augmented (center response) only during the transient period of depression of contractile responsiveness produced after osmolarity was increased. Responses in the center and at the right were obtained at 5 and 45 minutes, respectively, after osmolarity was increased (using sucrose). B: Similar augmentation of potassium-induced relaxation (center response) was demonstrated by simply reducing the level of drug-induced contractile tension by using a lower agonist concentration (see Fig. 2). Responses were obtained in the sequence shown from left to right.

10 mm Hg even in the vessel strips in which resting tension was relaxed when [K]o or osmolarity was increased, i.e., 50% of the coronary arterial strips.

Hypoxic-induced depression of drug-induced contractile tension was observed in nearly all of the strips tested in this study. In a representative group of strips, using 0.25-0.5 μM acetylcholine for 18 coronary arterial strips and 0.001-0.2 μM epinephrine for 43 deep femoral arterial strips, decreasing Po2 from 100 mm Hg to 10 mm Hg produced a mean fall in tension of 34.7 ± 4.6% (SE) in the coronary arterial strips and 38.5 ± 2.5% in the deep femoral arterial strips. This hypoxic depression of contractile tension was not relieved until Po2 was returned to higher levels. These effects of hypoxia on contractile tension were not altered significantly by changing to and subsequently testing in a salt solution with a [K]o of 5.88 mm and an osmolarity of 334 milliosmoles/liter.

**COMBINED EFFECTS OF HYPOXIA, HIGH EXTRACELLULAR POTASSIUM CONCENTRATION, AND HYPEROSMOLARITY**

Table 3 shows that hypoxia had no effect on the degree of relaxation and the duration of recovery of tension associated with increasing [K]o or osmolarity during agonist-induced responses. These experiments were conducted under conditions in which the amplitude of the test contractile responses at normoxia (Po2 of 100 mm Hg) and hypoxia (Po2 of 10 mm Hg) were maintained constant. In other words, if the responsiveness to agonist was depressed by hypoxia alone, testing for the effects of high [K]o and hyperosmolarity during hypoxia was done after the agonist concentration had been titrated upward so that the tension of the test response under hypoxia was the same magnitude as that under normoxia. If testing was carried out without adjusting for direct hypoxic depression of contractile tension (Fig. 9A), relaxation produced by high [K]o and hyperosmolarity was greater than that produced at hypoxia without depression, i.e., with the agonist concentration titrated upward. This additive interaction between hypoxia and the change to high [K]o and hyperosmolarity could be mimicked simply by reducing agonist concentration and thereby producing a similarly reduced contractile response for testing. As shown in Figure 9B, the relaxation produced by the combined change to high [K]o and hyperosmolarity under these conditions was greater than that produced at the higher contractile tension level.

**TABLE 3**

<table>
<thead>
<tr>
<th>Test condition</th>
<th>Coronary arterial strips</th>
<th>Deep femoral arterial strips</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relaxation (%)</td>
<td>Recovery time (minutes) N</td>
</tr>
<tr>
<td>High [K]o</td>
<td>36 ± 6.6</td>
<td>5.1 ± 0.22 8</td>
</tr>
<tr>
<td>High [K]o + hypoxia</td>
<td>37 ± 8</td>
<td>5.3 ± 0.36 8</td>
</tr>
<tr>
<td>Hyperosmolarity</td>
<td>46 ± 1.6</td>
<td>&gt;15 11</td>
</tr>
<tr>
<td>Hyperosmolarity + hypoxia</td>
<td>46 ± 2.7</td>
<td>&gt;15 11</td>
</tr>
<tr>
<td>High [K]o + hyperosmolarity</td>
<td>93 ± 4.3</td>
<td>9.9 ± 0.57 11</td>
</tr>
<tr>
<td>High [K]o + hyperosmolarity +</td>
<td>97 ± 3</td>
<td>9 ± 0.57 11</td>
</tr>
<tr>
<td>hypoxia</td>
<td></td>
<td></td>
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</tbody>
</table>

Data were obtained and are presented as discussed for Table 1. The effects of three different test conditions (elevated [K]o, hyperosmolarity and elevated [K]o + hyperosmolarity) were studied in each strip at a Po2 of 100 mm Hg and a Po2 of 10 mm Hg. To keep contractile tension the same for testing at both oxygen levels, the concentration of agonist was increased during hypoxia.
These concentrations of plasma produced contractile responses in both vessel types which were more than 50% of the maximum contraction produced by the other agonists used. Also, two experiments were done using histamine, which served as an agonist for both vessel types. The individual and combined effects of high \([K_o]\) and hyperosmolality, and hypoxia on contractile tension produced by these two agonists were essentially the same as those described for acetylcholine-induced and epinephrine-induced contractile tension.

Three experiments (three coronary and six deep femoral arterial strips) were carried out using 1-2% plasma (final bath concentration) as the agonist. These concentrations of plasma produced contractile responses in both vessel types which were more than 50% of the maximum contraction produced by the other agonists used. Also, two experiments (four coronary and two deep femoral arterial strips) were done using histamine, which served as an agonist for both vessel types. The individual and combined effects of high \([K_o]\), hyperosmolality, and hypoxia on contractile tension produced by these two agonists were essentially the same as those described for acetylcholine-induced and epinephrine-induced contractile tension.

Discussion

The investigator using isolated vessel preparations must make every effort to study them under conditions in which responsiveness mimics that existing in situ. Most often, the responsiveness of vasculature studied in vitro at least qualitatively confirms that observed in the intact vasculature. Occasionally, however, unexpected responses are seen which may, indeed, lead one to suggest that “studies of this nature should be considered with great care before the results are extrapolated to intact animals” (3). However, these same data may well represent a unique opportunity which can be exploited by the investigator to learn more about those aspects of the local environment that could prove critical for normal function in situ of the smooth muscle cells. The current study may serve as an example of this point.

The concentration of potassium in rabbit plasma is approximately 6 mM (4). Yet, with a \([K_o]\) of 6 mM in the tissue bath medium, we were not able to observe potassium-induced relaxation of strips taken from either coronary or deep femoral vessels of the rabbit (5). Only by reducing \([K_o]\) to levels no higher than 4 mM could this relaxation be produced. We have no direct evidence to explain this phenomenon. It is interesting to note that the intracellular potassium concentration ([K]i) of isolated vascular smooth muscle cells may be only approximately 50% of [K]i for cells in situ (6). If contractile responsiveness of vascular smooth muscle depends on the ratio of [K]i to [K]o (5, 7-9), perhaps the [K]i in the tissue bath medium should be dropped to approximately 3 mM, i.e., to a value also approximately 50% of the [K]i found in situ. Following this line of reasoning, we chose to carry out the present study with control [K]i equal to 3.18 mM on the basis of the tentative hypothesis that, with respect to vascular responsiveness to abrupt changes in [K]i, the ratio of [K]i to [K]o is more important than [K]o alone for making the isolated vessel preparation a representative model of “normal” responsiveness under conditions in situ. The values of either [K]i or the ratio of [K]i to [K]o may be less important for obtaining normal responsiveness to other chemical changes such as the shift from normal osmolarity to hyperosmolality.

Under the conditions chosen, the present study confirms other studies showing that vascular tone falls in response to hyperosmolality tested both in vivo (10-20) and in vitro (14, 21-23) and to elevated potassium concentration tested both in vivo (17, 18, 24-33) and in vitro (9, 21, 34-39). None of these earlier studies using isolated vessel preparations (9, 14, 21-23, 34-39) dealt with vasculature from skeletal muscle and only two (35, 37) dealt with isolated coronary vasculature. Our data also confirm the finding that the immediate fall in vascular tone in situ (18) produced by elevating potassium and osmolarity together is greater than that produced by either condition imposed separately, i.e., they demonstrate an additive interaction. It appears, therefore, that the in situ effects of potas-
sium and osmolarity alone or together can be accounted for entirely at the level of the vascular smooth muscle cells. Our data also demonstrate that the additive interaction between these conditions is not the result of a specific interaction but rather appears to be an effect that can be accounted for solely on the basis of a nonspecific depression of vascular tone; in other words, the relaxing effect produced by either elevated \([K]_o\) or hyperosmolarity can be augmented by any condition that results in depression of background vascular tone, e.g., by reducing the agonist concentration used to produce the active tension. The finding that the inhibition of vascular tone produced in vitro by hyperosmolarity and particularly by elevated \([K]_o\) was not sustained in duration confirms recent observations reported by other workers using intact vessel preparations. These workers have observed that vasodilation produced by either elevated potassium or hyperosmolarity can wane despite sustained elevation of \([K]_o\) (40) or osmolarity (10, 15, 16). The present data suggest that their observations can be explained on the basis of a direct mechanism involving only an interaction between potassium or hyperosmolarity and the vascular smooth muscle cells. More importantly, the data obtained from the present isolated vessel studies represent evidence in support of the suggestion that, although the combined effect of elevated \([K]_o\) and hyperosmolarity acting directly on the vascular smooth muscle cells can account for the onset and the very early period of vasodilation associated with active hyperemia (but, see ref. 41), this combination does not account for physiologically sustained vasodilation of intact vasculature within skeletal and cardiac muscle during prolonged exercise even if potassium and osmotic levels remain elevated throughout the duration of hyperemia. In fact, on the basis of our data and that of Brace et al. (40) using intact vasculature, one would expect that the direct effects on the vasculature of a sustained elevation in potassium concentration would ultimately be vasoconstriction rather than vasodilation. However, with respect to osmotic changes, our observations in conjunction with observations made on intact vasculature (15, 19) suggest that sustained hyperosmolarity by itself may act directly on the vascular smooth muscle cells to produce vasodilation of variable duration without any delayed vasoconstrictor effect.

Much previous work has been carried out using both intact (17, 18, 32, 33, 42–50) and isolated large vessel (51–56) preparations which has led to the suggestion that the vascular tone of the systemic circulation, which presumably includes that of the small resistance vessels, is depressed by hypoxia. The results of the present study support this suggestion at least with regard to the effects of severe hypoxia (\(P_o_2\) of 10 mm Hg) on small arteries isolated from rabbit cardiac and skeletal muscle. Furthermore, when vascular tone was depressed by severe hypoxia in the present study, the degree and the duration of the tension relaxation-recovery response pattern produced by elevated \([K]_o\) or hyperosmolarity were augmented compared with the response patterns to these chemical changes carried out under normoxic conditions (\(P_o_2\) of 100 mm Hg). These later observations confirm the finding that hypoxia augments the vasodilatory effect on intact vasculature produced by both elevated \([K]_o\) and hyperosmolarity (18). Since this additive interaction was not observed when the vascular tone of the isolated vessels did not (or was not allowed to) fall on exposure to hypoxia, it is concluded that any influence exerted by hypoxia on vascular responsiveness to changes in \([K]_o\) or osmolarity can be accounted for by a nonspecific effect on vascular tone similar to that discussed earlier regarding the additive interaction between high potassium and hyperosmolarity.

If one wishes to extrapolate the present observations concerning oxygen back to the intact vasculature, it should first be noted that, since the smallest of the vessel samples used in this study probably lacked vasa vasorum, the normal supply of oxygen to the media of these vessels was by diffusion alone and that probably this diffusion of oxygen was at least as good under isolated conditions as it was in situ. This observation implies that conditions within the walls of these vessels, which may or may not be analogous to the "anoxic core" condition described for larger vessel samples (57), were similar in vitro and in vivo and that the data obtained from isolated small vessel samples also apply for the same vessels in situ under the same conditions of extreme hypoxia. On the basis of these considerations, our data can be interpreted to indicate that hypoxia of sufficient degree can itself account for sustained vasodilation by acting directly on small vessels in cardiac and skeletal muscle of the rabbit. They also suggest that the degree of initial vasodilation produced by the combined effects of increased \([K]_o\), hyperosmolarity and hypoxia acting directly on the vasculature might be greater than those produced by the presence of any one or two of these conditions (but, see ref. 58 with respect to oxygen and the early
onset of active hyperemia). However, sustained vasodilation produced by all three conditions together would probably be no greater than that produced by hypoxia alone.

Two points must be kept in mind regarding these experiments conducted on isolated vasculature. The data relate to the interactions of only three of the many possible factors that could contribute to local regulation of vascular tone, and they relate only to the direct interaction of these three factors with smooth muscle cells of the vessel wall. It is certainly possible that elevated [K\textsubscript{i}], or hyperosmolarity could contribute to sustained vasodilation by acting directly on the vascular smooth muscle cells in conjunction with other vasoactive factors that have not been considered in this study or by acting on the surrounding parenchymal tissue so as to trigger the release of an unknown intermediary substance also having vasoactive properties.

This investigation supports studies carried out in situ which have led to the currently accepted opinion that the vasodilation associated with active hyperemia is the result of the interaction of several local chemical factors (16, 18). It further provides an explicit description of a nonspecific additive interaction among high [K\textsubscript{i}], hyperosmolarity, and hypoxia and shows that this interaction can be accounted for strictly at the level of the vascular smooth muscle cells without involvement of the surrounding parenchymal tissue. Although it appears that all three of these conditions, if present, can act to initiate vasodilation in either cardiac or skeletal muscle at the onset of exercise, this study suggests the possibility that hypoxia is the only one among them which can account for sustained vasodilation by a direct interaction with the smooth muscle cells of at least the small arteries. Since the two oxygen levels, i.e., 10 mm Hg and 100 mm Hg, used in this study are both outside the physiological range for small arteries in situ (59), the study does not directly help to answer the question of whether the normal fall in local Po\textsubscript{2} within cardiac and skeletal muscle during prolonged exercise contributes importantly to the associated sustained active hyperemia. Data must be obtained concerning (1) the intrinsic sensitivity of these small arteries to variations in Po\textsubscript{2} within the physiological range and, perhaps most important, (2) this same intrinsic sensitivity of the smaller resistance segments of the microvasculature. It will be important to see how future studies conducted on preparations both in situ and in vitro will direct our thinking on the interaction between hypoxia and certain cellular metabolites, e.g., adenosine, which, like hypoxia, also demonstrate sustained inhibitory activity on vascular tone (60) and have been seriously considered to have an important role in the local control of vascular tone in situ (61).

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Circulation Research, Vol. 35, November 1974
LOCAL CONTROL OF VASCULAR TONE


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