Effects of oxygen tension on energetics of cultured vascular smooth muscle

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Received 19 January 2001; accepted in final form 19 March 2002

Lindqvist, Anders, Karl Dreja, Karl Swärd, and Per Hellstrand. Effects of oxygen tension on energetics of cultured vascular smooth muscle. Am J Physiol Heart Circ Physiol 283: H110–H117, 2002; 10.1152/ajpheart.00040.2001.—Chronic hypoxia is a clinically important condition known to cause vascular abnormalities. To investigate the cellular mechanisms involved, we kept rings of a rat tail artery for 4 days in hypoxic culture (HC) or normoxic culture (NC) (PO2 = 14 vs. 110 mmHg) and then measured contractility, oxygen consumption (JO2), and lactate production (Jlac) in oxygenated medium. Compared with fresh rings, basal ATP turnover (JATP) was decreased in HC, but not in NC, with a shift from oxidative to glycolytic metabolism. JO2 during mitochondrial uncoupling was reduced by HC but not by NC. Glycogen stores were increased 40-fold by HC and 4-fold by NC. Maximum tension in response to norepinephrine and the JO2 versus tension relationship (JO2 vs. high K+-elicited force) were unaffected by either HC or NC. Force transients in response to caffeine were increased in HC, whereas intracellular Ca2+ wave activity during adrenergic stimulation was decreased. Protein synthesis rate was reduced by HC. The results show that long-term hypoxia depresses basal energy turnover, impairs mitochondrial capacity, and alters Ca2+ homeostasis, but does not affect contractile energetics. These alterations may form a basis for vascular damage by chronic hypoxia.

LOWERED OXYGEN TENSION (hypoxia) has generally been found to rapidly decrease contractile force of vascular smooth muscle, a reaction likely to be important for the local regulation of blood flow in response to tissue metabolic demands (see Ref. 30 for a review). Several processes involved in excitation and contraction may be affected by hypoxia, e.g., Ca2+ homeostasis, ion channel properties, and the cross-bridge interaction. However, the long-term effects of lowered PO2 on contractility and metabolic patterns are less well known, despite the clinical importance of vascular alterations associated with tissue hypoxia and ischemia. In particular, chronic hypoxia has been implicated in the development of atherosclerosis as a result of intimal thickening or occlusion of vascular supply to the arterial wall (3, 20).

Experimentally induced chronic hypoxia in vivo has been shown to attenuate vasoreactivity to various contractile agents (1, 5, 12), to decrease production of d-myo-inositol 1,4,5-trisphosphate (32), to decrease Ca2+ sensitivity of arterial myofilaments (35), and to decrease collagen synthesis (10). In cultured arterial tissue, α1B-adrenoreceptor mRNA was increased in response to long-term hypoxia (8). Cells exposed to prolonged hypoxia adapt by inhibition of ATP-consuming processes, e.g., protein synthesis and Na+/K+ pumping (11, 25). However, limited information is available regarding metabolically dependent processes that may be inhibited by chronic hypoxia in intact smooth muscle.

To investigate chronic effects of hypoxia on vascular smooth muscle, we utilized a tissue culture model previously established to preserve phenotypic differentiation and contractility of vascular preparations over several days (16). Oxygen consumption (JO2) and lactate production (Jlac) were determined under basal and stimulated conditions in cultured as well as fresh preparations, allowing determination of basal metabolic rates and energetic tension cost. Responses to hypoxia and metabolic inhibitors, as well as the occurrence and frequency of intracellular Ca2+ waves, were studied to investigate the functional consequences of metabolic adaptation to culture under normoxia or hypoxia.

MATERIALS AND METHODS

Organ culture. Female Sprague-Dawley rats weighing ~200 g were euthanized by cervical dislocation. The experimental procedures were approved by the Animal Ethics Committee of Lund University. A 5-cm segment of the tail artery, beginning ~1 cm distal to the radix, was dissected free under sterile conditions and transferred to a petri dish containing culture medium composed of Dulbecco’s modified Eagle’s medium and Ham’s F-12 (1:1; Biochrom; Berlin, Germany) with the addition of antibiotics (50 μg/ml penicillin and 50 μg/ml streptomycin). The vessel segment had a diameter of ~0.5 mm along its entire length and was cut into 0.5-mm-thick rings under a dissection microscope. The rings were transferred to culture dishes containing medium as described above, with supplements as indicated for the respective experiments. The dishes were placed in a water-jacketed cell incubator at 37°C for 4 days. Rings cultured under hypoxic conditions were incubated under 95% N2-5% CO2. The PO2 in the medium after 4 days was 14 mmHg, as determined with an oxygen electrode. To estimate the PO2 in the tissue, we

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first calculated the drop in PO₂ from the liquid surface to the tissue, giving a PO₂ at the tissue surface of ~7 mmHg. The PO₂ distribution across the arterial wall was then calculated as previously described (33) for an inside radius of 150 μm and an outer radius of 250 μm. Assuming J₀₂ in the tissue was 6 × 10⁻⁵ ml O₂ ml⁻¹ s⁻¹ and Krogh’s constant of 3.17 × 10⁻¹⁰ ml O₂ cm⁻¹ mmHg⁻¹ s⁻¹, the PO₂ in the middle of the arterial wall was found to be ~3 mmHg.

Force registration. Arterial rings were relaxed for 20 min in a nominally Ca²⁺-free Krebs solution. The rings were then mounted on parallel stainless steel wires (diameter 0.25 mm), one of which was connected to a force transducer (model AE 801, SensoNor; Horten, Norway) and the other to an adjustable support. Preparations were immersed in a modified Krebs solution in exchangeable Plexiglas cups (0.4 ml) fitted into a thermostated metal block (37°C) under normoxic conditions. The solution had the following composition (in mM): NaCl 118.35, KCl 5.9, CaCl₂ 2.5, MgCl₂ 1.2, HEPES 11.6, pH 7.4, glucose 5.6, EGTA. The rings were allowed to equilibrate for 20 min, stretched to optimal length as described previously (16), and activated twice with high-K⁺ solution, prepared by isomolar exchange of NaCl for KCl. Concentration-response curves to norepinephrine (NE; 1 nM-30 μM) were obtained in a cumulative manner.

The contractile responses under metabolic challenge by hypoxia (PO₂ = 12 mmHg) or the metabolic inhibitors rotenone and 2,4-dinitrophenol (DNP) were determined as follows. Arterial rings were stimulated with NE (1 μM) for 5 min and allowed to relax for 23 min. Two minutes before the next contraction, the inhibitor was added to the bathing solution, and the rings were then stimulated with NE for 5 min in the presence of the inhibitor. The relative change in tension development between the two consecutive contractions was determined. In one set of experiments the effects of glucose removal were examined. Arterial rings were stimulated with NE (1 μM) for 5 min, followed by relaxation for 25 min. This cycle was repeated five times. During the first stimulation, glucose was present in the medium. Five minutes before the next stimulation, glucose was removed from the medium and was absent during the remainder of the experiment. The decrease in tension development in response to successive stimulations with NE was normalized to the first contraction. Responses to caffeine (20 mM) were measured after 2 min in Ca²⁺-free medium containing 1 mM EGTA.

Measurements of metabolic rates. J₀₂ was recorded using a method described by Lövgren and Hellstrand (17). Modified Krebs solution containing antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin) was introduced into a glass chamber (0.25 ml vol) from a reservoir where it was equilibrated with air. The decrease in PO₂ with time was recorded using a polarographic O₂ electrode (ESchweiler; Kiel, Germany). Background J₀₂ was recorded after each experiment. In cases where a background was detected, it was subtracted from the measured J₀₂. All measurements of J₀₂ were carried out under normoxic conditions.

For determination of Jₐₐₜ, the tissue was incubated at 37°C for 10 min in 0.6 ml of modified Krebs solution with antibiotics equilibrated in air. The tissue was removed and weighed, and the solution samples stored in a freezer (−20°C) until analyzed. The samples were then analyzed for lactate with the use of enzymatic fluorimetric methods, as described by Lowry and Passoneau (18). The rate of ATP turnover (Jₐₐₜ) was calculated from the relationship Jₐₐₜ = 6.42 J₀₂ + 1.25 Jₐₜ (see Ref. 22). For determination of glycolytic products, tissue was frozen in liquid nitrogen and homogenized in 0.02 M HCl. Glycolytic products were determined enzymatically (18). Briefly, glycolysis was degraded to glucose-6-P with the use of phosphorylase debrancher complex A and P-glucomutase. Glucose-6-P was transformed to 6-P-glucose-6-lactone using glucose-6-P-dehydrogenase and reduced NADP fluorescence was determined.

All metabolic rates are related to wet weight, determined after gentle blotting of the tissue on filter paper. To investigate the effects of culture conditions on tissue composition, tail arteries were placed in a closed Eppendorf vial after blotting, weighed, cultured, and then weighed again using the same procedure. The samples were then freeze-dried for determination of dry weight and extracted for total protein analysis.

Confocal microscopy. For detection of intracellular Ca²⁺ concentration ([Ca²⁺])ₐₐₜ, waves, rings were mounted inside-out on thin glass capillaries (diameter 250 μm) and incubated with the [Ca²⁺]ₐₐₜ indicator Fluo-4-acetoxyethyl ester (10 μM) and pluronic F-127 (0.05%, both from Molecular Probes) at room temperature for 80 min. Exciting light was at 488 nm and emitted light was detected at >505 nm using a laser scanning confocal microscope (model 510LSM, Zeiss; Jena, Germany). Preparations were washed in modified Krebs solution (22°C) for at least 15 min before NE (0.1 μM) was added. Images were obtained every 0.37 s for 30 s, and wave activity was assessed in all distinguishable cells with the use of Zeiss 510LSM software.

Protein synthesis. Protein synthesis in freshly dissected or cultured arterial rings was determined by measuring L-[4,5-³H]leucine (Amersham Pharmacia Biotech; Little Chalfont, UK) incorporation. Segments of rat tail artery were cultured as described above. After 4 days, the segments were transferred to normoxic medium and allowed to equilibrate for 1 h. The preparations were incubated with 1 μCi/ml of L-[4,5-³H]leucine for 1 h. The reaction was stopped by placement of the culture dishes on ice. The tissue was frozen in liquid nitrogen, transferred to test tubes containing 5 mM NaOH, and homogenized by sonication. An aliquot of the homogenate was precipitated with 5% trichloroacetic acid and centrifuged at 13,200 g for 2 min at 4°C. The pellet was washed once with trichloroacetic acid and dissolved in Soluene (Packard Instrument, UK). A liquid scintillation cocktail (Optiphase HiSafe2; Wallac Scintillation Products) was added and the radioactivity was measured using a scintillation counter (model LS6500, Beckman Coulter; Fullerton, CA). Total protein content was determined using a Bio-Rad protein assay.

Statistics. Summarized data are expressed as means ± SE. Student's t-test was used to evaluate statistical significance. For multiple comparisons, one-way ANOVA was used. P < 0.05 was considered statistically significant.

RESULTS

Effects of hypoxic culture on force development of arterial rings. Culture of tail arterial rings in serum-free medium does not affect maximal force in response to NE, as demonstrated earlier (15). Rings cultured under continuous hypoxia (PO₂ = 14 mmHg) showed unimpaired maximal force response in oxygenated medium compared with rings cultured under normoxia (Fig. IA), although the EC₅₀ for NE was increased (179 ± 27 vs. 73 ± 15 nM, P < 0.05, n = 3). In contrast to NE-induced responses, force development in high-K⁺ solution is decreased after culture, irrespective of conditions used (Fig. IB). Tension transients in response to caffeine (20 mM) in Ca²⁺-free
solution were larger after hypoxic than after normoxic culture (Fig. 1C).

**Metabolism of freshly dissected and cultured arterial rings.** $J_{O_2}$, measured under normoxic conditions, was greater in Ca$^{2+}$-containing solution than in Ca$^{2+}$-free normal solution in both fresh and cultured arterial rings, and higher still during depolarization and contraction in high-K$^+$ solution (Fig. 2A). Under both basal and stimulated conditions, $J_{O_2}$ values were lower in cultured than in fresh rings, and particularly the basal $J_{O_2}$ was depressed after culture under hypoxia. However, the slope of $J_{O_2}$ versus tension developed in response to high-K$^+$ solution is unaltered by culture (freshly dissected 0.032 ± 0.003, culture normoxia 0.034 ± 0.004, and culture hypoxia 0.033 ± 0.003, n.s., n = 5 in all groups, Fig. 3A). Maximal mitochondrial capacity was determined using stimulation with the uncoupler DNP. $J_{O_2}$ in the presence of DNP (30 μM) was similar after normoxic culture as in fresh tissue, but reduced after hypoxic culture (Fig. 3B).

In fresh preparations, $J_{\text{lac}}$ showed the same pattern as $J_{O_2}$, with successive increases over the basal value upon addition of Ca$^{2+}$ and during high-K$^+$ contraction. In cultured rings, on the other hand, basal $J_{\text{lac}}$ was greater than in the fresh rings and showed no alteration with addition of Ca$^{2+}$ or contraction (Fig. 2B). $J_{\text{lac}}$ values were lower after hypoxic culture than after normoxic culture.

The $J_{\text{ATP}}$ was calculated from $J_{O_2}$ and $J_{\text{lac}}$ (see MATERIALS AND METHODS and Table 1). It is seen that in rings cultured in normoxia, the lower $J_{O_2}$ relative to fresh rings is offset by the higher $J_{\text{lac}}$ so that basal $J_{\text{ATP}}$ is the same. In rings cultured under hypoxic conditions, the basal ATP consumption is decreased by ~40%.

**Effects of acute hypoxia on $J_{\text{lac}}$.** $J_{\text{lac}}$ was determined under basal conditions and during stimulation with NE (Fig. 4). As in the experiments shown in Fig. 2, basal $J_{\text{lac}}$ values were considerably greater in cultured than in fresh rings. In freshly dissected rings and in rings cultured under normoxia, $J_{\text{lac}}$ increased only marginally during stimulation with NE (1 μM), but in rings cultured under hypoxia the increase was greater and statistically significant. When rings were stimulated with NE in hypoxic solution, $J_{\text{lac}}$ increased in all groups of preparations, showing the presence of a Pasteur effect, i.e., increased glycolysis compensating for the lack of oxidative metabolism (Fig. 4).

**Contractility during metabolic inhibition and glucose deprivation.** Contractile properties of fresh and cultured preparations were determined under hypoxia.
and in the presence of several metabolic inhibitors. Hypoxia only marginally affected NE-induced tension development in the cultured rings, while in fresh preparations tension was reduced by 40% (Fig. 5). Mitochondrial inhibition by rotenone (10 μM) had a somewhat greater effect, and DNP (30 μM) was still more effective. Both inhibitors had significantly smaller effects on cultured than on fresh rings.

Glycogen contents were ~4× greater in rings cultured in normoxia than in fresh rings, whereas in rings cultured under hypoxia the increase was ~40-fold (Fig. 6A). Sensitivity to glucose removal was tested by the loss of force in repeated contractions in glucose-free medium. Rings cultured under normoxia were more sensitive than fresh rings, which in turn were more sensitive than rings cultured under hypoxia (Fig. 6B).

Intracellular \( \text{Ca}^{2+} \) waves. We tested whether temporal \( \text{Ca}^{2+} \) coding in the form of waves was affected by culture, and whether this would correlate with the altered responsiveness to metabolic inhibition. With the use of confocal microscopy, intracellular \( \text{Ca}^{2+} \) waves were detected in cells within intact rings loaded with Fluo-4. While wave activity was low under resting conditions, it increased during stimulation by NE. To avoid confluence of individual waves and thus allow calculation of wave frequency, an intermediate concentration of NE (0.1 μM) was used, representing close to \( EC_{50} \) for cultured preparations (see above) but clearly below.

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<th>Table 1. ( J_{\text{ATP}} ) in freshly dissected and cultured tail artery</th>
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<td>( J_{\text{ATP}} ) in high-\text{K}⁺ solution, μmol·g⁻¹·min⁻¹</td>
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Values are means ± SE. \( J_{\text{ATP}} \), rate of ATP consumption.
below EC\(_{50}\) for freshly prepared rings (0.6 \(\mu M\)), in which the sensitivity to exogenous NE is influenced by prejunctional uptake (16, 19). Despite the relatively greater level of stimulation by NE compared with fresh tissue, the number of cells with wave activity in cultured preparations and also the frequency of waves in individual cells, was lower by \(\sim 50\%\) (Fig. 7). Culture under hypoxia further decreased both parameters of wave generation.

**Protein synthesis in cultured arterial rings.** Protein synthesis in arterial rings cultured for 4 days was studied as an incorporation of L-[4,5-\(^3\)H]leucine in normoxic medium. Rings cultured under hypoxia showed a lower incorporation of leucine compared with rings cultured under normoxic conditions, which in turn showed lower incorporation than freshly dissected rings (Fig. 8).

To evaluate the influence of protein synthesis on metabolic rates, the effect of cycloheximide (10 \(\mu M\)) on \(J_{O_2}\) was determined. In fresh rings, cycloheximide decreased basal \(J_{O_2}\) by 25\% \((P < 0.05, n = 3)\), while there was no significant effect after either normoxic or hypoxic culture \((n = 3)\).

The effects of culture conditions on weight and protein contents of the tissue were determined. Arteries were weighed before and after culture as described in MATERIALS AND METHODS. The arteries were then freeze-dried to obtain dry weight. As shown in Table 2, the wet weight decreased during culture, in agreement with earlier results (16). However, the weight loss was \(\sim 20\%\) smaller after hypoxic culture. This reflects a true difference in protein contents because the dry-to-wet weight ratio did not differ between fresh and cultured preparations. Protein determinations verified that the protein contents-to-dry weight ratio was the same after hypoxic and normoxic culture (data not shown).
DISCUSSION

Exposure of vascular smooth muscle tissue to maintained hypoxia for several days was found to cause reduced basal $J_{\text{ATP}}$ and rate of protein synthesis. Under our hypoxic conditions (14 mmHg), and assuming a $P_{O_2}$ of 7 mmHg at the surface of the vessel, we calculate that all cells are near or above the mitochondrial limiting $P_{O_2}$ of 1–2 mmHg. Although this neither supports nor excludes a potential limitation on mitochondrial O$_2$ supply, other mechanisms may be involved. Whereas basal $J_{\text{ATP}}$ had decreased by 40% when measured in oxygenated medium following hypoxic culture, it was the same as in fresh tissue after normoxic culture. However, in common with hypoxic culture, the regulation of glycolysis was altered, with an increased rate of glycolytic relative to oxidative metabolism. 

Expression of glucose transporters and glycolytic enzymes is under the control of hypoxia-inducible factor 1, which regulates a multitude of genes by a molecular-sensing mechanism that is rapidly becoming understood (reviewed in Ref. 26). It thus appears that conditions during normoxic culture are sufficient to activate this program to some extent, although responses to hypoxic culture are more pronounced. The diminished relaxation response to hypoxia seen after both normoxic and hypoxic culture may reflect the shift from oxidative to glycolytic metabolism. Thorne et al. (31) recently reported a diminished relaxation response to hypoxia after 24-h normoxic culture of porcine coronary artery.

Total protein contents were better preserved during hypoxic than during normoxic culture. It is notable that environmental stress, such as hypoxia, generally causes decreased protein turnover, especially evident as a slowing of protein degradation (9). However, both during hypoxic and normoxic culture conditions protein was lost, indicating that degradation is faster than synthesis, although contractility is preserved over the presently used time span. Because no growth promoter (i.e., fetal calf serum) was added, protein synthesis was expected to be slow. Furthermore, we applied no mechanical tension, a factor that has been shown to increase the rate of protein synthesis and to promote growth during culture of vascular tissue (2, 34). Electron microscopy of tail arterial rings cultured under the normoxic conditions used here shows essentially normal morphology (16), and thus the loss of protein is

| Table 2. Relative wet weight and dry-to-wet weight ratios in freshly dissected and cultured tail artery |
|-----------------------------------------------|--------------|-----------------|
| Relative Wet Weight | Dry Weight/Wet Weight |
| Freshly dissected | 1.0 | 0.229 ± 0.007 |
| Culture normoxia | 0.777 ± 0.013 | 0.227 ± 0.007 |
| Culture hypoxia | 0.827 ± 0.013a | 0.237 ± 0.007 |

Values are means ± SE; $n = 4$ for each group. $^aP < 0.05$, culture under hypoxic vs. normoxic conditions.
not associated with a change in cellularity of the tissue, as also suggested by the maintained basal $J_{ATP}$ shown here. The energetic tension cost, evaluated as $J_{O_2}$ relative to developed force for high-K$^+$ stimulation, was closely similar among all three experimental groups, suggesting that the basic mechanisms of force generation had not been affected by culture, either hypoxic or normoxic. $J_{O_2}$ has been shown to correlate with force development in vascular tissue, whereas $J_{lac}$ primarily reflects energy turnover associated with ion pumping (23), which may have been affected by culture (see below).

Maximum contractile responses to NE were unaffected after hypoxic culture, whereas responses to high-K$^+$ depolarization were reduced. This pattern is also seen when preparations cultured under normoxia are compared with freshly prepared tissue (Ref. 15 and this study). Culture of arterial tissue downregulates the expression of voltage-activated Ca$^{2+}$ channels (6), which may explain the decreased high-K$^+$-induced force. This would not affect NE-induced force to the same extent because NE causes sensitization to Ca$^{2+}$ (7). Although the effect of chronic hypoxia on the expression of voltage-dependent Ca$^{2+}$ channels has not been examined in systemic vascular smooth muscle cells, it is interesting that a decreased Ca$^{2+}$ transient was found after hypoxic culture of cardiac myocytes and suggested to be part of a protective response against Ca$^{2+}$ overload (27).

NE stimulates glycogenolysis and thus mobilizes energy from glycolysis, evident as an increased $J_{lac}$. Rings cultured under normoxia lost force faster than fresh rings when repeatedly stimulated with NE in glucose-free medium, consistent with the greater reliance on glycolytic metabolism after culture. In contrast, rings cultured under hypoxia were markedly more resistant to glucose-free medium than freshly dissected rings, correlating with the dramatically increased glycogen stores in this tissue. A similar phenomenon has been described in cardiac myocytes cultured under hypoxia and subsequently reoxygenated (27), although the increase in glycogen stores was only about twofold, in contrast to the 40-fold increase seen here in the vascular rings. Overall, the metabolic reactions to chronic hypoxia in cultured vessels resemble alterations occurring in atherosclerosis (3).

We (7) have previously shown that intracellular Ca$^{2+}$ stores are upregulated during culture of the tail artery. The present results suggest that culture under hypoxia further augments these stores, because it increased the magnitude of caffeine-induced contractions, dependent on Ca$^{2+}$ release from intracellular stores via ryanodine receptors. This suggests that the uptake of Ca$^{2+}$ into the sarcoplasmic reticulum is increased, implying increased demand of energy for pump activity. This is consistent with the increase in glycolytic rate in cultured vascular preparations. Mitochondrial uncoupling produced maximal rates of $J_{O_2}$, which were essentially unaffected by culture under normoxia, but significantly decreased after hypoxic culture. All experimental groups also exhibited Ca$^{2+}$-dependent control of mitochondrial function (21).

Inhibition of mitochondrial oxidative phosphorylation by rotenone and uncoupling of ATP production from oxidative phosphorylation using DNP produced similar responses as hypoxia in fresh and cultured preparations, although the loss of contractile force was greater, particularly with DNP. Adrenergic stimulation promotes generation of intracellular Ca$^{2+}$ transients in the form of recurrent waves, as demonstrated both in arterial (13) and venous (24) smooth muscle. In Xenopus laevis oocytes, this kind of temporal Ca$^{2+}$-coding appears to be regulated by mitochondrial metabolism (14), and in the tail artery we have demonstrated that intracellular Ca$^{2+}$ wave activity is altered by rotenone, such that a pattern of low-frequency, high-amplitude waves is shifted to lower amplitude and higher frequency (29). In contrast, DNP eliminates all wave activity. The present results show that following organ culture, the number of cells with wave activity is decreased, as well as the frequency of waves in individual cells. These alterations were enhanced after hypoxic culture, suggesting that they are related to mitochondrial inhibition. The implications of this finding need to be explored further, but may be considered to include a role of wave activity in regulating several cellular processes. In addition to a possible role of intracellular Ca$^{2+}$ waves in force production (13, 24), Ca$^{2+}$ waves may influence protein synthesis because intermittent Ca$^{2+}$ transients have been shown to be more efficient than sustained elevations in activating Ca$^{2+}$-dependent transcription factors in cultured cells (4) and native smooth muscle (28).

The present study shows that exposure of vascular smooth muscle to chronic hypoxia shifts cellular metabolism toward glycolytic energy production, increases glycogen stores, and confers resistance to acute hypoxia. These effects are accompanied by altered cellular Ca$^{2+}$ handling and decreased protein synthesis, whereas the energetic cost of contraction is unaffected. Because this study was performed on intact differentiated vascular tissue, its results point to deviations from normal function that may be early responses to chronic hypoxia of the vascular wall, a known risk factor for vascular disease.

We thank Ina Nordström for performing lactate and glycogen determinations and Dr. Juris Galvanovski for calculations of oxygen pressure distribution.

The study was supported by the Swedish Medical Research Council Project 04X-28.

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