Energy Cost of Membrane Depolarization in Hog Carotid Artery

John W. Peterson and Elvira Glück

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SUMMARY. Past studies have shown that during stable stepwise activations of vascular smooth muscle with varying concentrations of high-K+, both cytoplasmic free-Ca++ and membrane depolarization vary. On the other hand, during stepwise activations with varying concentrations of external Ca++, in the presence of constant external depolarizing high-K+, cytoplasmic free-Ca++ varies, while membrane depolarization remains relatively constant. In this study, the rates at which suprabasal energy metabolism (oxygen consumption and lactic acid production) increased with increasing isometric tension maintenance were measured under both circumstances. Suprabasal energy metabolism with increasing membrane depolarization (increasing external K+ ) exceeded that with constant depolarizing-[K+] and varying Ca++ by less than 2.5% at all levels of activation, which was not statistically significant (P > 0.70). We conclude therefore that the steady state metabolic energy cost of membrane depolarization per se during contractile activity in vascular smooth muscle from a tonic conducting vessel (hog carotid artery) is negligible. Although the possibility cannot be excluded, we find no metabolic evidence that increased cytoplasmic free-Ca++ itself activates an ATPase associated with Ca++ sequestration and/or extrusion beyond that present in the relaxed state. Activation of hog carotid artery with an isosmotic K+-for-Na+-substituted medium fails to stimulate aerobic glycolysis at all levels of K+ substitution. Experiments were performed at the muscle length optimal for isometric tension generation and at 37°C. (Circ Res 50: 839–847, 1982)
from the adventitia by the methods described by Glück and Paul (1977) and by Driska et al. (1981) which avoid excessive stretching. Tissues then were returned to cold storage until used, usually within 3 days. No alterations in sensitivity to agonists, isometric tension generation, or metabolic parameters were noted in comparing artery strips stored 1, 2, or 3 days. Similar insensitivity to cold storage has been noted in studies with other vascular smooth muscle preparations (Paul and Peterson, 1975; Sparks and Bohr, 1962).

The muscular media strips were mounted between stainless steel compression clips in a thermostated (37° ± 0.2°C) oxygen consumption chamber (volume 14.8 ml) which permitted the ready exchange of bathing solutions, addition of substrates and drugs, the periodic removal of small (0.9 ml) samples of the bathing media for chemical analysis, the adjustment of muscle length, and determination of isometric tension. The chamber and construction details have been described previously (Paul and Peterson, 1975). In this series of experiments on 24 arteries, the muscular media strips (mean wet weight 59.1 ± 19.1 mg) averaged 0.6 (±0.04) mm thick by 8.4 (±1.6) mm long between the clips under a resting tension which averaged 150 (±75) gwt/cm². Force was measured in gram weight (gwt), which is equivalent to 980 dyne (9.8 × 10⁻³ Newton). Tissue cross-section area was taken as wet weight divided by tissue length, assuming a density equal to 1.0.

Solutions

Krebs-Henseleit physiological saline was used as the normal bathing medium (Na⁺-PSS) throughout, containing (in mm): NaCl, 118; KCl, 5.32; NaH₂PO₄, 1.54; NaHCO₃, 24.9; MgSO₄, 1.19; and CaCl₂, 2.53. The pH was 7.4 at 37°C when gassed with 50% O₂/45% N₂/5% CO₂, which was the normal gas composition for the experiments and assures adequate oxygenation (Glück and Paul, 1977). A high-K⁺ saline (K⁺-PSS) was made by complete substitution of K⁺ salts for all Na⁺ salts. High-K⁺ activating solutions then were adjusted isosmotically for K⁺ and Na⁺ content by mixing volumes of Na⁺-PSS and K⁺-PSS. All saline also contained 0.01 mM Na₂EDTA to chelate heavy metal ion impurities, as well as 100 mg/liter streptomycin sulfate and 300 mg/liter penicillin G to counter bacterial action. Glucose was added to the chamber from a 360 mM stock solution to a final concentration of 5 mM with each solution change.

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Results

Basal Metabolism

Table 1 gives the mean observed metabolic properties for the hog carotid arteries used in these experiments, illustrating again the extensive involvement of aerobic glycolysis in smooth muscle metabolism (Peterson and Paul, 1974a; Glück and Paul, 1977; Kroeger, 1977; Hellstrand, 1977). Typically, 20-25% of basal ATP production is provided by lactic acid production, even in well-oxygenated vascular smooth muscle preparations. Whereas our mean, weight-normalized basal values are slightly higher (~20%) than those reported by Glück and Paul (1977) for a larger series of experiments with the same artery preparation, the difference is not significant (P ~ 0.2). Our

<table>
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<tr>
<th>Table 1</th>
<th>Mean Basal Metabolic Properties in Isolated Hog Carotid Artery</th>
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<tr>
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<td>Mean</td>
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<tr>
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</table>

All values: µmol/min per g wet artery. n = number of determinations.
protocols differed primarily only in that a brief maximal contraction with both high-K⁺ and histamine preceded the metabolic measurements. No statistically significant differences in basal oxygen consumption rate (Jₒₒ), lactic acid production rate (Jₐₐ), or computed ATP utilization rate (Jₐₐₜₚ) measured before and after the first experimental stimulation were detected. The higher basal metabolic rates per gram wet artery segment in our series may correspond with the significantly greater isometric force per unit cross-section area developed (~twice that reported by Glick and Paul for high-K⁺ activation), perhaps indicating that more muscular artery segments were studied in this series.

Isometric Tension Development

We compared a variety of activating conditions for isometric tension development. Although norepinephrine (NE) has been reported to give large stable contractions in carotid arteries from American hogs (Herlihy, 1972), below 10⁻⁶ m, it failed to elicit any contractile response, and supramaximal doses at 5 × 10⁻⁵ m gave comparatively small (~50% of high-K⁺ response) and transient contractions with carotid arteries from German hogs, and therefore was not used as a regular stimulant. To avoid effects of a possible Na⁺/K⁺-ATPase inhibition in the complete absence of Na⁺, we sought to find the level of K⁺ substitution in the complete absence of Na⁺, which is well above the levels reported to be vasodilatory (Alta and Altura, 1978). Figure 2A illustrates that a further increase in [K⁺]₀ to ~50 mm (30% K⁺ substitution) gives, on average, nearly the full response. Figure 2B shows that the isometric tension response to high K⁺ is linearly proportional to log[K⁺]₀ up to ~85% of the maximal K⁺ response. The proportionality is similar to that reported in other vascular smooth muscles (Berner et al., 1980). Since membrane potential has also been shown to vary linearly with log[K⁺]₀ in the same range of external [K⁺] (cf Horn, 1978), this suggests a direct correlation between membrane depolarization and isometric tension development. K⁺-activated tension is maximal by 75 mm K⁺₀, and further increases in K⁺₀ activate, on average, no further tension development. In sequential comparisons of tension development in 50% and 100% K⁺-PSS in 10 artery segments, the ratio of tensions developed averaged 1.01 (±0.02 SEM).

Figure 1 further shows the effect of such graded K⁺ contractures on the oxygen consumption rate (upper panel), and complete reversibility of the procedure as indicated by the reproducibility of the maximal tension and oxygen consumption values in 50% K⁺-PSS + HIST. At the end of each Jₒₒ measurement period, samples of the bathing media were taken and frozen until subsequent analysis of lactic acid content. Dilution effects due to removal and replacement of the bathing media sample are accounted for by computation. Maximum tension generation was typically greater than 1.5 kgwt/cm² with no correction for nonmuscular cross-section area. This value is comparable to that determined for American hog carotid artery (Murphy et al., 1974) and other VSM preparations (cf Paul, 1980).

Figure 3 is a plot of the observed oxygen consumption and lactic acid production rates (Jₒₒ and Jₐₐ) measured under conditions of increasing [K⁺]₀ substitution in five arteries. The order of graded K⁺ concentrations was varied (rather than always simply increasing, monotonically, as shown in Figure 1) to avoid possible sequential or hysteric effects. The scales for Jₒₒ and Jₐₐ in Figure 3 are constructed so as to represent the relative contribution of each process to the total ATP production rate (Jₐₐₜₚ) and mean basal rates (±50) for the particular set of arteries used in these experiments are indicated by the brackets. Several features should be noted. There is some compensatory nonlinearity seen in both the Jₒₒ and Jₐₐ data. For small contractions (below ~35 mm K⁺₀), both Jₒₒ and Jₐₐ are seen to increase, although the increase in Jₐₐ is not statistically significant when compared directly with basal Jₐₐ. Beyond ~35 mm K⁺₀, however, Jₒₒ increases more rapidly with increasing activation, whereas Jₐₐ remains essentially con-
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**FIGURE 1.** Upper: Composite record of the oxygen electrode output recorded during each contraction with varying \([K^+]_o\) in a single experiment. The slope of the trace is the oxygen consumption rate, which is seen to rapidly achieve a steady value following the replacement of one bathing media with another (which also restores chamber \(pO_2\) to the initial value). Chamber \(pO_2\) was not permitted to decline by more than 10% following this procedure. Lower: Record of the isometric tension during the protocol described in text for a single artery segment. Force was calibrated in gram weight \((gwt = 9.8 \times 10^{-3} N)\). Upper and lower panel from the same experiment.

**FIGURE 2.** A: Stable isometric tension development as a function of external \(K^+\) and \(Na^+\) concentration is shown. Each symbol type represents data from a single artery segment \((n = 7)\). \(K^+-\)activated tension, which averaged 1.18 \((\pm 0.08)\) kgwt/cm² in 18 arteries, was measured before and after the graded-\(K^+\) series. \(K^+-\)activated tensions at other \([K^+]_o\)'s are expressed relative to the greater of the two values measured at 50% \(K^+-\)PSS \(78 \text{ mM } K^+, 72 \text{ mM } Na^+\). Ten artery segments were activated sequentially in 50% and 100% \(K^+-\)PSS. The mean ratio of tensions developed \((\pm SEM)\) is shown by the brackets and is not statistically different from unity. B: Mean isometric tension \((\pm SEM)\) is plotted against the external \([K^+]_o\) on a logarithmic scale. Tensions development up to ~85% of maximum \(K^+-\)activated tension is linearly proportional to \(log[K^+]_o\).
Simultaneously measured values of oxygen consumption rate (J_o2, upper) and lactic acid production (J_LA, lower) in five arteries are plotted as a function of the isometric tension (ΔP0) developed in response to varying concentrations of [K+]. Basal metabolic rates in Na+-PSS were measured before and after the graded-K+ series; occasionally during. The mean basal values (± standard deviation) are indicated by the brackets. Where both J_o2 and J_LA are plotted in μmol/min per g wet artery, the scales are in the ratio 6.42 to 1.25, so as to illustrate visually the contribution of each to total ATP production. Each symbol type represents an artery so that individual trends may be noted. Solid lines were fit by eye.

In Figure 4, the J_o2 and J_LA data have been combined (as described in Methods) for each individual pair of measurements to compute the total steady state metabolic energy utilization in terms of ATP, and are plotted as the suprabasal rate of ATP utilization vs. maintained active isometric tension (ΔP0). The non-linear J_o2 and J_LA data of Figure 3 are seen to be exactly compensatory in terms of maintaining a linear correlation between increased energy utilization and isometric tension maintenance (linear regression coefficient r = 0.94). That the regression line passes through the origin is consistent with the premise that no detectable changes in basal metabolic rates occurred during the K+-activated contractions. Direct comparisons of basal rates measured before, during, and after a series of K+ contractions indicate that nothing more than a slow, time-dependent decline of basal metabolism on the order of 4 (±2) %/hr occurs following the described protocol, a value similar to that reported by Chace and Odessey (1981).

The slope of the suprabasal ΔJ_ATP-isometric tension relation (0.407 ± 0.031 μmol ATP/min per g wet artery per kgwt/cm² tension maintained) is similar to the isometric energy cost measured in a variety of other smooth muscles (cf Paul 1980 for review). The increasing energy utilization with increasing contraction represents, under these conditions, primarily the sum of steady state energy utilizations due to increasing membrane depolarization, increasing actomyosin ATPase, and increasing intracellular [Ca++].

**Metabolic Energy Utilization during Graded Ca++ in High-K+ Contractions**

In the experiments above, the level of activation was varied through graded membrane depolarization by altering (presumably in a similar graded fashion) the membrane Ca++ permeability at constant external [Ca++]. Alternatively, one could vary the activation in constant depolarizing high-K+ solution by varying the external [Ca++]]. For these experiments, the following protocol was used. Artery segments were relaxed in normal Na+-PSS, then incubated 20-30 minutes in Ca++-free Na+-PSS with 0.5 mM EGTA added; following which the effect of Ca++-free 50% K+-PSS with 0.5 mM EGTA was determined. This procedure did not alter resting tension and completely abolished (>99%) any contractile response, as expected. The effects on metabolism of this sequence of first removing external Ca++, then K+-depolarizing in the ab-
sence of Ca++ is shown in Table 2. Depleting external Ca++ in the normally polarized artery segment (column 2) exerts no statistically detectable (P > 0.6) influence on overall metabolic rates relative to the respective values in normal Na+-PSS (column 1), whether by comparing mean values (as given in Table 2) or by paired comparison (which in no case exceeded 15%). Subsequent K+ depolarization of the membrane in the absence of Ca++ has no effect on oxygen consumption rate, although a small (~15%) but statistically significant (P < 0.08) increase in lactic acid production rate was noted by paired difference analysis (column 3). Whereas such an effect could be consistent with the premise that Na+/K+-ATPase is coupled to aerobic glycolysis, the effect on overall energy metabolism (JATP) is small (~5%) and not significant (P = 0.70).

Following Ca++-depletion, as described, [Ca++]₀ then was varied in the bathing 50% K+-PSS solution, resulting in stable graded isometric tension responses much like those shown in Figure 1 for graded K+. The recovery of tension relative to the initial 50% K+-PSS response as a function of [Ca++]₀ is shown in Figure 5. Recovery of isometric tension was subject to wide variation (recovery at 1.0 mM Ca++-depletion ranged from 47% to 98%) and often incomplete, even when long equilibration times were allowed (up to 1 hour). This “non-recovered” K+-activated tension was not due to irreversible losses in tension-generating capacity (cf Peterson and Paul 1974b), since addition of histamine to the incompletely recovered artery segment yielded a total tension identical to that observed before Ca++ deple- tion with 50% K+-PSS + HIST (cf also Fig. 8). The metabolic response to increasing [Ca++]₀ and increasing tension development following Ca++-depletion is shown in Figure 6 for data from five arteries. As Ca++ is progressively restored, J0ₙ is seen to in-
crease in strict linear accord with isometric tension maintenance. JLA, on the other hand, shows great variability and no discernible trend with increasing [Ca++]₀ and maintained K+ depolarization, other than to be slightly elevated above the range of Ca++-free JLA values with no tension development (columns 2 and 3 of Table 2). One feature to point out is that, whereas simultaneous Ca++ depletion and membrane depolarization per se had no effect on J0ₙ and only a slight effect on JLA (column 3 of Table 2), the initial Ca++ restoration (typically 0.1 mM) following Ca++-depletion caused a significant decline in J0ₙ and an increase in JLA. These changes apparently were maintained throughout the balance of the Ca++ restoration, since the basal shifts are also manifest in the relaxed basal metabolic values (i.e., in Na+-PSS) after Ca++ had been fully restored. (In spontaneously active rat portal vein, Hellstrand (1977) has reported the exact opposite effect, namely, that J0ₙ abruptly increased upon Ca++ restoration to greater than 0.05 mm in similar experiments; JLA was not measured.) The effects of this Ca++-depletion-restoration cycle on basal J0ₙ and JLA are compensatory, so that—on average—basal JATP following a Ca++-depletion-restoration cycle was not substantially altered, but shifted toward a greater contribution from aerobic glycolysis. This appears to be no ready explanation as to why Ca++ restoration should depress oxygen consumption, when Ca++ depletion itself had essentially no effect.

In Figure 7, suprabasal JATP (taken relative to the

![FIGURE 5. Tension development (expressed relative to the initial maximal K+-activated tension) is shown as a function of the external [Ca++] with 50% K+-PSS activation following Ca++-depletion by the protocol described in text. Each symbol type represents data from one artery segment. In 50% K+-PSS with 0.5 mM EGTA and no added Ca++, contractile response averaged ~0.5% of the initial maximal tension with K+ activation.]

**TABLE 2**

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<table>
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<tr>
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All values: [mole/min per g wet artery; error determinations are standard deviations; number of determinations shown in parentheses. Column 3 shows the mean change in metabolic rates observed with 6 artery segments compared to their respective values under the conditions of column 2; significance tested by paired variate analysis and Student’s t-test.]
basal measured at the end of each experiment to account for the small basal shift described above) is plotted against the isometric tension developed in response to increasing \([Ca^{++}]_0\). The strict linear relationship, and the fact that the regression line passes through the origin, indicate that the shift in basal metabolism described above occurred in response to the initial \(Ca^{++}\) restoration (rather than progressively with increasing \([Ca^{++}]_0\)), and was then subsequently stable. The observed correlation between suprabasal energy utilization rate and active isometric tension maintenance (0.398 ± 0.010 μmol ATP/min per g wet artery per kgwt/cm² tension) is essentially identical to that determined in the case of graded \(K^+\) contractions. In three artery segments, both graded \(K^+\) and graded \(Ca^{++}\) in 50% \(K^+\)-PSS experiments were performed. An example of such pairwise comparisons is shown in Figure 8, in which the suprabasal \(J_{ATP}\) data are seen to be exactly co-linear, as were the data in all three such comparisons. This illustrates that the agreement in metabolic response to the two sets of activating conditions is not only statistically equivalent in a large population of arteries, but is also clearly seen in each individual sample. As long as careful attention was paid to alterations in basal metabolic rates, this equivalence was not dependent on the order in which graded \(K^+\) and graded \(Ca^{++}\) series were performed.

**Discussion**

Varying levels of cytoplasmic free-\(Ca^{++}\) activate actomyosin ATPase and, consequently, isometric tension...
Ca\(^{++}\) levels. One expects in this case the actomyosin 5'-monophosphate (cAMP) may modulate the re-

### Figure 8.

Suprabasal \(J_{\text{ATP}}\) data such as that presented in Figures 4 and 7 for multiple experiments are shown for two experiments in a single artery segment. The segment was first taken through a series of contractions in graded-K\(^{+}\) solutions (circles), then Ca\(^{++}\)-depletes according to normal protocol, and tension restored by grade replacement of Ca\(^{++}\) in 50% K\(^{+}\)-PSS (squares). Note that while Ca\(^{++}\)-replacement restored only about 60% of the initial maximal K\(^{+}\)-activated tension, the subsequent addition of histamine agrees well with an earlier measurement in 50% K\(^{+}\)-PSS + HIST (barred symbols). The metabolic responses to graded K\(^{+}\) and graded Ca\(^{++}\) in high-K\(^{+}\) are seen to be co-linear.

### Discussion

**Energy Metabolism in Hog Carotid Artery**

Suprabasal energy metabolism at the same isometric tension would reflect primarily differences in "activation energy utilization" per se, due to the different modes of activation used.

In the experiments reported here in which cytoplasmic free-Ca\(^{++}\) was varied by varying the transmembrane Ca\(^{++}\) flux, we found that suprabasal energy metabolism was the same function of isometric tension, regardless of whether the extracellular K\(^{+}\) concentration varied or was held constant. Consequently, we conclude that the increase in energy expenditure in response to membrane depolarization per se is negligible in comparison to the overall energy utilization. Since, under these conditions, the principal factor varied was cytoplasmic free-Ca\(^{++}\), the increased energy expenditure with increasing Ca\(^{++}\) is due to Ca\(^{++}\)-activated actomyosin ATPase and other ATP-requiring processes related to Ca\(^{++}\). Of these other Ca\(^{++}\)-related processes, presumably those related to maintaining Ca\(^{++}\) homeostasis could be a large part. Depolarizing the membrane in the absence of Ca\(^{++}\) does not significantly alter energy metabolism (Table 2), as was also the finding in the spontaneously active rat portal vein (Hellstrand, 1977). In contrast to our results here, however, where Ca\(^{++}\)-depletion-restoration causes a slight depression of \(J_{0}\), and no change in \(J_{\text{ATP}}\), Hellstrand observed that \(J_{0}\) increased abruptly when low levels of Ca\(^{++}\) insufficient to activate contraction were restored to the Ca\(^{++}\)-depleted rat portal vein. If this increased metabolism represents energy-dependent Ca\(^{++}\)-activated Ca\(^{++}\) sequestration and/or extrusion in the portal vein, then the absence of a similar effect in hog carotid artery (Fig. 6 and 7) suggests the absence of substantial Ca\(^{++}\) sequestration and/or extrusion which are activated by increased intracellular Ca\(^{++}\) alone. Whereas Ca\(^{++}\) activates the Ca\(^{++}\)-Mg\(^{++}\) ATPase of Ca\(^{++}\)-accumulating microsomal fractions from tonic arteries, the effect was small (cf Jones, 1980). It is interesting to speculate that this is a fundamental difference in the underlying mechanisms of Ca\(^{++}\) homeostasis in spontaneously active and graded tonic vessels. On the other hand, it is possible that such Ca\(^{++}\)-activated processes also increase in linear accord with isometric tension in hog carotid artery, in which case our experiments could not detect them.

Under both sets of activating conditions described above, oxygen consumption rate correlates closely (and, for the most part, linearly) with isometric tension maintenance, whereas aerobic glycolysis does not. This suggests that aerobic ATP production is closely coupled to the actomyosin ATPase (be it spatially or otherwise), while glycolytic ATP production is not. Aerobic glycolysis in hog carotid artery is not activated (<15%, Table 2) by increasing membrane depolarization or by increasing cytoplasmic free-Ca\(^{++}\) alone (Fig. 6); but is slightly activated by a Ca\(^{++}\)-depletion-restoration cycle in K\(^{+}\)-substituted medium. However, this may reflect a compensatory response of aerobic glycolysis to some impairment of mitochondrial activity as much as an effect on aerobic glycolysis itself. That membrane-depolarization per se does not substantially alter energy metabolism in tonic vascular smooth muscle perhaps reflects quantitatively a paucity of Na\(^{+}\)/K\(^{+}\)-ATPase, since Kroeger (1977) observed a substantial increase in both \(J_{0}\) and \(J_{\text{LA}}\) upon K\(^{+}\) depolarization of Ca\(^{++}\)-depleted myo-mitun. The failure of substituted high-K\(^{+}\) solution to activate aerobic glycolysis in hog carotid artery is not due to a K\(^{+}\) inhibition, since Paul et al. (1979) found that activation by hypertonic KCl addition (80 mm) does stimulate lactic acid production. This suggests the possibility that aerobic glycolysis in tonic

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- Conti and Adelstein, 1980
- Mrwa et al., 1979
- Hellstrand, 1977
- Jones, 1980
- Paul et al., 1979
vascular smooth muscle is strongly affected by relatively small (~30 mM) changes in extracellular [Na\(^+\)] when the membrane is depolarized.

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Energy cost of membrane depolarization in hog carotid artery.
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