Energy Cost of Membrane Depolarization in Hog Carotid Artery

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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 development 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)

IN THIS and a subsequent report (Peterson 1982), we compare the metabolic energy cost of isometric tension maintenance in the isolated hog carotid artery under three sets of activating conditions by which the level of isometric tension development may be altered in a stable graded fashion. In all three conditions, activation with high extracellular K⁺ is used. It is well-established that activation of vascular smooth muscle (VSM) contraction by high-K⁺ solution occurs through membrane depolarization (Burnstock and Straub, 1958; Somlyo et al., 1969), yielding increased transmembrane permeability to extracellular Ca⁺⁺ (Briggs, 1962; Hudgins and Weiss, 1968) as well as an exchange/release of some loosely bound fraction of intracellular Ca⁺⁺ (Hinke, 1965; Weiss, 1975). An inward flux of extracellular Ca⁺⁺ maintains an elevated cytoplasmic free-Ca⁺⁺ level (Van Breeman et al., 1973; Karaki and Weiss, 1980), thus activating a stable contracture (Bohr, 1963; Bohr et al., 1978) as well as, undoubtedly, other Ca⁺⁺-sensitive processes.

In previous studies on intact isolated arteries and veins, we have investigated the energy metabolism of VSM contractility and identified quantitatively those components of overall energy metabolism associated with the direct maintenance of isometric tension (i.e., actomyosin ATPase) and those associated with tension-development processes or "activation" per se (Peterson and Paul, 1974a; Paul and Peterson, 1975 1977). Activation energy metabolism was measured as that part of overall suprabasal energy metabolism that remains when tension-dependent metabolism in a fully activated smooth muscle preparation is abolished, and represents the metabolic energy requirements of processes such as increased transmembrane ion pumping, phosphorylation/dephosphorylation cycles, and energy-dependent intracellular Ca⁺⁺ translocation.

The studies described below were undertaken to examine in more detail the energetics of particular processes which constitute some part of the "activation energy." In this report, we focus on the metabolic energy cost due to membrane depolarization per se, whereas a subsequent report (Peterson 1982) examines the excess metabolic energy cost of hormonal activation. In whole frog skeletal muscle, it has long been known that membrane depolarization by even moderately elevated extracellular [K⁺] results in a dramatic (up to 20-fold) increase in resting energy metabolism (Solandt, 1936) which is not completely reversed even after 6 hours of exposure. In arterial smooth muscle from a tonic conducting vessel, after the energy cost of contractility is accounted for, the metabolic energy cost of prolonged membrane depolarization is found to be negligible.

Methods

Artery Preparation and Apparatus

Hog carotid arteries were obtained at a commercial slaughterhouse within 20 minutes of exsanguination, rinsed of blood and cleaned of loose adventitia, and transported in room temperature physiological saline solution (PSS). Tissues then were cooled to 5°C and strips of media separated...
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

Kreb's-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 streptomyacin 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. Pharmacological agonists (histamine hydrochloride, norepinephrine bitartrate) were obtained from Sigma and were also added to the chamber in small volumes (0.1 ml) from stock solutions. All solutions were passed through Millipore sterilizing filters immediately prior to use in the chamber—again, to minimize bacterial oxidation. In the external Ca²⁺-dependence experiments, both Na⁺- and K⁺-PSS were prepared as described above, except that CaCl₂ was deleted. In some cases, 0.5 mm Na₂EGTA was added. Calcium concentration was varied in the appropriate saline then by mixing volumes of normal and Ca²⁺-free K⁺- and Na⁺-PSS.

Metabolic Measurements

Oxygen consumption and lactic acid production rates were measured as described by Peterson and Paul (1974a). Lactic acid measurements were made by discrete sampling of the bathing media at various time intervals and are not therefore as precise as the continuous recordings of oxygen consumption provided by the polarographic electrode method, since they represent time-averaged rates of production (typically 15 min). The rates of metabolic ATP production are computed from the steady state oxygen consumption and lactic acid production rates using the stoichiometric ratios of 6.42 mol ATP/mol O₂ consumed and 1.25 mol ATP/mol lactic acid produced. The computation of these conversion factors and bounds on the error introduced by their use have been presented (Peterson and Paul, 1974a). In the steady state, when intracellular ATP levels are constant, ATP production rates can be equated to ATP utilization rates. Paul and colleagues have found that the content of high-energy phosphates (ATP + PCr) is constant as soon as 30 seconds after activation of hog carotid artery (personal communication).

Experimental Protocol

After being removed from cold storage and mounted in the stainless steel holder clips under cold Na⁺-PSS, the tissues were allowed 1–1.5 hours to equilibrate at room temperature in Na⁺-PSS with 5 mm glucose. Arteries then were mounted in the oxygen consumption chamber and length adjusted to give a peak stress of 1–1.5 kgw/cm², which rapidly diminished. Such stretches then were repeated after stretch-relaxation every 20–30 minutes until, at 90 minutes, the resting tension was adjusted to about 150 gwt/cm², which has been found to be near the level for optimal tension development in hog carotid artery (Herlihy and Murphy, 1973). The tissues then were maximally activated with high-K⁺ saline containing 10⁻⁵ m histamine for 10–15 minutes, returned to Na⁺-PSS, and allowed at least 30 minutes to relax. This procedure (especially the addition of histamine to the initial high-K⁺ contracting solution) was adopted prior to each experiment with the intent of permitting internal Ca²⁺ levels and sequestration sites to achieve normal resting values. Only after this attempt at thorough mechanical, metabolic, and ionic equilibration were the various experimental protocols begun (see text). Resting tension in Na⁺-PSS was stable throughout the experiments, usually about 8 hours.

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 I | Mean Basal Metabolic Properties in Isolated Hog Carotid Artery |
|----------------|-------------------|-------------------|-------------------|
|               | Mean          | ±0.004           | ±0.014           | ±0.031           | ±0.198          | ±0.198          |
| J₀₂            | 0.093         | 0.003            | 0.139            | 0.748            | 1.595           | 1.595           |
| Jₐₐ            | 0.139         | ±0.004           | ±0.014           | ±0.031           | ±0.198          | ±0.198          |
| ATP            | 0.748         | ±0.031           | ±0.198           | ±0.198           | ±0.198          | ±0.198          |
| Jₐₐ/J₀₂        | 1.595         | ±0.031           | ±0.198           | ±0.198           | ±0.198          | ±0.198          |

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 (Jo), lactic acid production rate (JLA), or computed ATP utilization rate (JATP) 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⁺ for Na⁺ substitution just sufficient to maximize the high-K⁺ isometric contraction. A comparison of mean stable isometric tension developed in response to 100% K⁺-substituted saline (n = 10), and 50% K⁺-substituted saline (72 mM Na⁺, 77.5 mM K⁺; n = 22) gave no significant difference (P < 0.45) (cf also Fig. 2). For these reasons, 50% K⁺-substituted saline (50% K⁺-PSS) was used throughout as the standard maximal high-K⁺ activating solution.

To find conditions which maximized isometric tension, we determined the additive actions of norepinephrine and histamine. Whereas the addition of NE failed to increase isometric tension development in the already K⁺-depolarized artery (P > 0.65, n = 6), added histamine (10⁻⁵ M) elicited an additional tension increment which averaged 42% (± 8% SEM, n = 13) of K⁺-activated tension (cf Fig. 1). That this was an additive effect is evidenced by the fact that isometric tension development in 10⁻⁵ M histamine alone did not differ from the K⁺-activated tension. The ratio of histamine-activated to K⁺-activated isometric tensions averaged 1.11 ± 0.19 SEM in four paired comparisons, although tension maintenance was less stable with histamine alone. This maximal tension response in 50% K⁺-PSS plus histamine (HIST)-activating solution was used as the criterion for reproducibility of tension generation throughout the experiments.

Metabolic Energy Utilization during Graded K⁺ Contractions

By varying the ratio of Na⁺ to K⁺ in the incubating PSS, a series of stable graded isometric tensions can be generated in the isolated hog carotid artery (Fig. 1, lower panel), presumably due to graded membrane depolarization leading to graded increases in membrane Ca²⁺ permeability at fixed external [Ca²⁺]₀ (cf Bolton, 1979). The apparent threshold for K⁺-activated tension development is just below 10% K⁺ substitution (20 mM K⁺₀, 130 mM Na⁺₀), which is well above the levels reported to be vasodilatory (Altura 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⁺₀ 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⁺ contractions 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 J₀, 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 (J₀ and J_LA) 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 hysteretic effects. The scales for J₀ and J_LA in Figure 3 are constructed so as to represent the relative contribution of each process to the total ATP production rate (JATP) and mean basal rates (± SD) 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 J₀ and J_LA data. For small contractions (below ~35 mM K⁺₀), both J₀ and J_LA are seen to increase, although the increase in J_LA is not statistically significant when compared directly with basal J_LA. Beyond ~35 mM K⁺₀ however, J₀ increases more rapidly with increasing activation, whereas J_LA remains essentially con-
stant or perhaps declines slightly (although, again, the declining trend cannot be verified statistically with respect to the basal \( J_L \) values in Na\(^+\)-PSS). In any case, with K\(^+\) activation at all levels of K\(^+\) for Na\(^+\)-substitution, there is no substantial increase in aerobic glycolysis beyond the basal metabolic level. At 100% K\(^+\)-substitution, Glück and Paul (1977) reported no increase in lactic acid production rate above basal, and frequently observed a distinct inhibition. In further studies of this effect, Paul et al. (1979) have postulated that aerobic glycolysis is coupled to the Na\(^+\)/K\(^+\)-ATPase, thus explaining the inhibition of

![Diagram](image1)

**Figure 1.** Upper: Composite record of the oxygen electrode output recorded during each contraction with varying \([\text{K}^+]_o\) 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.

![Diagram](image2)

**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/cm\(^2\) in 18 arteries, was measured before and after the graded-K\(^+\) series. K\(^+\)-activated tensions at other [K\(^+\)]\(s\) are expressed relative to the greater of the two values measured at 50% K\(^+\)-PSS (78 mM K\(^+\), 72 mM Na\(^+\)). Ten artery segments were activated sequentially in 50% and 100% K\(^+\)-PSS. The mean ratio of tensions developed (\( \pm \text{SEM} \)) is shown by the brackets and is not statistically different from unity. B: Mean isometric tension (\( \pm \text{SEM} \)) is plotted against the external [K\(^+\)] on a logarithmic scale. Tensions development up to \( \sim 85\% \) of maximum K\(^+\)-activated tension is linearly proportional to log[K\(^-\)].
aerobic glycolysis in the complete absence of Na⁺. The data above indicate, however, that lactic acid production fails to be stimulated by contractile activity, even at low values of activation by K⁺ in the presence of substantial Na⁺0.

In Figure 4, the Jₒ 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 (△Pₒ). The non-linear Jₒ 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 J_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 [Ca²⁺].

Metabolic Energy Utilization during Graded Ca²⁺ 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 Ca²⁺ permeability at constant external [Ca²⁺]. Alternatively, one could vary the activation in constant depolarizing high-K⁺ solution by varying the external [Ca²⁺]. For these experiments, the following protocol was used. Artery segments were relaxed in normal Na⁺-PSS, then incubated 20–30 minutes in Ca²⁺-free Na⁺-PSS with 0.5 mM EGTA added; following which the effect of Ca²⁺-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 Ca²⁺, 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++], 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++]o is shown in Figure 5. Recovery of isometric tension was subject to wide variation (recovery at 1.0 mM Ca++ following 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++ depletion with 50% K+-PSS + HIST (cf also Fig. 8).

The metabolic response to increasing [Ca++], and increasing tension development following Ca++ depletion is shown in Figure 6 for data from five arteries. As Ca++ is progressively restored, Jo, is seen to in-

<table>
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<th>TABLE 2</th>
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<td>Effect of Ca++ Depletion on Metabolic Rates in 50% K+-PSS</td>
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<tr>
<th></th>
<th>Normal</th>
<th>Ca++-free</th>
<th>Δ Metabolism</th>
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<tr>
<td></td>
<td>Na+-PSS</td>
<td>Na+-PSS</td>
<td>Ca++-free</td>
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<tr>
<td>2.5 mM Ca++</td>
<td>+ EGTA</td>
<td>+ EGTA</td>
<td>50% K+-PSS</td>
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<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
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<tr>
<td>JATP</td>
<td>0.726</td>
<td>0.709</td>
<td>0.025</td>
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<tr>
<td>±0.058</td>
<td>±0.036</td>
<td>±0.018</td>
<td>±0.08</td>
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<tr>
<td>JLA</td>
<td>0.113</td>
<td>0.133</td>
<td>0.019</td>
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<td>±0.041</td>
<td>±0.050</td>
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All values: μmole/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.

In Figure 7, suprabasal JATP (taken relative to the

crease in strict linear accord with isometric tension maintenance. JLA, on the other hand, showed great variability and no discernible trend with increasing [Ca++], 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 Jo, 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 Jo, and an increase in JLA. These changes apparently were than 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 Jo 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 Jo, 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. There 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 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.
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 \((0.398 \pm 0.010 \mu\text{mol ATP/min per g wet artery per kgwt/cm}^2\text{tension})\) 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 ten-
ATPase in the steady state to be the same. Differences, levels. One expects in this case the actomyosin ++
5'-monophosphate (cAMP) may modulate the re-
however, it has been found that cyclic adenosine 3',
membrane permeability at fixed [Ca
++
]o. Recently,

isometric tension reflects similar cytoplasmic free-
reported for K
++
 substitution in hog carotid artery stimulates only a
small and not significant increase in tissue cAMP
(Conti and Adelstein,
1980; Mrwa et al., 1979). Activation by K
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 for Na
+
/substituted
Ca
++
 is varied by varying the trans-
segment was first taken through a series of contractions in graded-K
* solutions (circles), then Ca
++
-depletion 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 histo-
mime 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.

**FIGURE 8.** Suprabasal J
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
++
depletion according to normal protocol, and tension restored by grade replacement of Ca
++
in 50% K
+/PSS (squares). Note that while Ca
++
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ion development to varying extents, regardless of whether intracellular free-Ca
++
is varied by varying [Ca
++
]o at fixed membrane permeability or by varying membrane permeability at fixed [Ca
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]o. Recently, however, it has been found that cyclic adenosine 3', 5'-monophosphate (cAMP) may modulate the re-
sonce to cytoplasmic free-Ca
++
 (Conti and Adelstein,
1980; Mrwa et al., 1979). Activation by K
* for Na
*/K
* substitution in hog carotid artery stimulates only a small and not significant increase in tissue cAMP
(Peterson 1982), whereas no effect on cAMP has been
reported for K
* depolarization of rabbit aorta (van
Breeman, 1976). Thus, it is reasonable to expect, under these conditions, that the same level of steady isometric tension reflects similar cytoplasmic free-
Ca
++
levels. One expects in this case the actomyosin ATPase in the steady state to be the same. Differences, then, in the 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 cyto-
plasmic free-Ca
++
 was varied by varying the trans-
membrane 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
ATP,
Hellstrand observed that J
0,
increased abruptly when low levels of Ca
++
in insufficient to ac-
tivate 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 processes 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
ATP upon K
* depolarization of Ca
++
depleted myo-
metinum. 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
vascular smooth muscle is strongly affected by relatively small (~30 mM) changes in extracellular [Na\(^+\)] when the membrane is depolarized.

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References


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