The Effects of Isoproterenol and Ouabain on Oxygen Consumption, Lactate Production, and the Activation of Phosphorylase in Coronary Artery Smooth Muscle

Richard J. Paul

SUMMARY. In previous work, oxidative metabolism in vascular smooth muscle was found to be correlated with isometric force; aerobic lactate production, however, was found to be substantial and correlated with Na-K transport processes (Paul et al., 1979; Paul, 1980). In this work, the role of glycogen phosphorylase in this unusual functional compartmentalization of vascular energy metabolism was investigated. Isometric force, oxygen consumption, lactate production, and the level of phosphorylase activity were measured in porcine coronary arterial segments. For comparison with previous studies, phosphorylase activity was also measured in rabbit aorta. Added potassium chloride induced a contracture in which oxygen consumption, lactate production, and phosphorylase activity all increased. Ouabain also induced a contracture, and an increase in oxygen consumption and phosphorylase activity. However, lactate production was inhibited. Isoproterenol, when added to a potassium chloride-induced contracture, elicited a relaxation in isometric force, and oxygen consumption returned to basal levels; however, lactate production and phosphorylase activity remained at the previously elevated levels. Isoproterenol alone had only marginal effects on all parameters studied. The results indicate that the role of phosphorylase in vascular metabolism is complex and unlikely to be a rate-limiting factor for the observed aerobic glycolysis. The high levels of phosphorylase activity observed in the absence of actomyosin interaction support a mechanism of β-adrenergic relaxation in which the sensitivity of actin-myosin interaction to calcium ion is decreased. (Circ Res 52: 683–690, 1983)
with Na-K transport in VSM. In particular, the effects of isoproterenol on \( J_{\text{Na}} \) and phosphorylase on both relaxed and stimulated coronary vessels were studied in detail, inasmuch as a major hypothesis concerning the mechanism of \( \beta \)-adrenergic relaxation involves stimulation of Na-K transport (Schied, et al., 1979). Preliminary reports of this work have been presented (Paul and Doerman, 1980; Paul et al., 1980).

**Methods**

**Tissue Preparation**

Hearts were collected within 45 minutes after slaughter from hogs of 60-90 kg, then were placed in physiological saline solution (PSS) and transported to the laboratory on ice. The right coronary artery (RC) was dissected from the heart, and loose fat and connective tissue were removed in cold PSS. Multiple segments (up to 10) approximately 0.5 cm long were cut from each artery. Typical dimensions of these segments were 20 mg blotted weight, 7.5 mm in circumference, and 0.5 ± 0.08 mm thick (which includes both media and adventitia). The segments were gently inverted with forceps so that the media was on the outside and adventitia inside to facilitate \( O_2 \) diffusion. One segment was mounted isometrically between a movable and fixed stainless steel wire (0.7 mm diameter), and this assembly was placed in the polarographic chamber (see below). The remaining segments from the same artery were cannulated on glass rods, the diameter of which was chosen to match the internal diameter of the unstimulated vessel so that the arterial segments were fixed isometrically at approximately the peak of their length-tension curve throughout the course of the experiments measuring lactate production and phosphorylase activity (see below). Before mounting, the segments were gently blotted and then weighed in a tared PSS to determine the wet weight. In some experiments, thoracic aorta from rabbits were used. Aorta were dissected after the rabbit had been killed by a cervical dislocation, and were treated in a manner similar to that described for the porcine coronary vessels. The dimensions of the rabbit aorta were approximately the same as the porcine coronary arteries.

**Solutions**

The physiological saline solution contained (mM): NaCl, 130; NaHCO\(_3\), 14.9; KCl, 4.7; KH\(_2\)PO\(_4\), 1.18; MgSO\(_4\), 1.17; CaCl\(_2\), 1.6; and glucose, 5.5. Solutions were gassed with mixtures containing 3% CO\(_2\), resulting in a pH between 7.3 and 7.4 at 37°C. To chelate heavy metal ion impurities, 0.01 mM EDTA was used. The PSS also contained 300 mg/liter penicillin G (500 U/ml) and 100 mg/liter streptomycin sulfate to retard bacterial growth. As additional checks to bacterial contamination, all solutions used were passed through Millipore sterilizing filters (0.45 \( \mu \)m). Modifications of this standard solution are detailed in the experimental section. Isoproterenol and ouabain (strophanthin-G) were of this standard solution are detailed in the experimental section. Isoproterenol and ouabain (strophanthin-G) were added in the amilgamator and allowed to thaw to 0°C. A further 350-\( \mu \)m aliquot of homogenization solution (0°C) was added and mixed by hand. After 5 minutes of extraction at 0°C, the Teflon capsule was placed in an Eppendorf microcentrifuge and spun at 15,000 g for 1-2 minutes. The supernatant was assayed for phosphorylase activity. The homogenization solution contained (mm): \( \beta \)-glycerophosphate, 20; NaF, 10; EDTA, 2; \( \beta \)-mercaptoethanol, 10; and 1 mg/ml bovine serum albumin; the pH of this solution was adjusted to 6.8 at room temperature with KOH.

Phosphorylase was assayed using an enzyme-linked procedure described by Hardman et al. (1965) as utilized by Namn (1971) for rabbit aorta. The activity is reported as micromoles of glucose 1-phosphate produced per minute at 30°C. This assay was modified in a similar fashion, as reported by Entman et al. (1976). In this modification, the reaction mixture contains the following: AMP of 2-4 mg/ml (rabbit aorta) appeared negligible. The inclusion of activated charcoal (0.1 mg/ml) was used to remove any endogenous tissue AMP on the phosphorylase activity at the tissue concentration by 5-fold had no significant effect on the activity ratio. This is in agreement with the results of Hardman et al. (1965); in their procedure, the glucose 1-phosphate produced is measured separately. The continuous method was chosen as its precision at low phosphorylase activities is greater.

An activity ratio for phosphorylase a was calculated as: activity:activity in the presence of 5'-adenosine monophosphate (AMP) at 1 mM. An example of the calibration of the assay and continuous measurement of NADPH fluorescence reflecting phosphorylase activity is shown in Figure 1. The relation between added AMP and phosphorylase activity was studied in several cases. The apparent \( K_m \) for AMP of between 0.01 and 0.02 mM (rabbit aorta) appeared somewhat lower than the value of 0.3 mM reported for skeletal muscle (Entman et al., 1976). The effects of endogenous tissue AMP on the phosphorylase activity at the tissue concentration used in the assay (2-4 mg/ml) were negligible. The inclusion of activated charcoal (0.1 mg/ml) in the homogenization medium and further dilution of the tissue concentration by 5-fold had no significant effect on the activity ratio. This is in agreement with the results of Namn (1971) and consistent with the apparent \( K_m \) and an approximate 600-fold dilution of tissue AMP in this assay.

**Mechanical Measurements**

Isometric measurements of force were made using a Statham (UC2) transducer (compliance = 0.2 \( \mu \)m/mN). The arterial cross-section area, used to normalize isometric force data, was estimated using the tissue weight and circumference (c), assuming a density (\( \rho \)) of 1.06 g/cm\(^3\) according to:

\[
A = \frac{2 \times \text{weight} \times 2 \pi \cdot c}{\rho \cdot \pi \cdot c}\]

**Mathematical Treatment of the Data**

Standard parametric statistics are used. A probability of less than 0.05 is taken as statistically significant.
PHOSPHORYLASE ACTIVITY

![Diagram of phosphorylase activity]

**Figure 1.** Measurement of phosphorylase activity. Left panel: ordinate - fluorescence (arbitrary units); abscissa - time. This panel shows the calibration of the output of the fluorimeter corresponding to the production of NADPH by the enzyme-linked assay system in response to the addition of 0.01 µmol glucose 1-phosphate. Right panel: ordinate and abscissa as described above. An aliquot of rabbit aorta extract (see Methods), corresponding to approximately 1.25 mg wet weight, was added to the assay system initiating the record. A steady state rate was obtained after several minutes and corresponds to the endogenous phosphorylase activity. At the arrow, 5'-AMP was added to bring its concentration in the reaction mixture to 2 mM. A new steady state rate is achieved corresponding to the total phosphorylase activity. An activity ratio is defined as the ratio of activity in the absence of 5'-AMP to that in the presence of 5'-AMP.

**Experimental Protocol**

Tissues were incubated for a minimum of 2 hours at 37°C before the first measurements of basal parameters were made. By this time, basalJo2 had attained a steady rate which was reproducible for approximately 8-12 hours after mounting. Tissue deterioration was judged by failure of Jo2 to return to basal levels after a test when the solution was returned to its normal values. Isometric force on the tissue in the polarographic chamber was increased in a stepwise fashion during the equilibration until a stable force of approximately 20 mN/mm² was achieved. Previous experiments had indicated that passive tension of this magnitude occurred at the length that was optimal for development of active force. When stimulated with added KCl (see below), both O2 consumption and isometric force were found to decrease if the O2 partial pressure decreased below a critical partial pressure of 0.08 atm (Paul et al., 1979; Paul, 1983b). O2 consumption and isometric force were found to be independent of the O2 partial pressure in experiments performed between air and 93% O2. The majority of experiments were thus conducted between an initial partial pressure of 0.4 atm and 0.25 atm, at which point the solution was changed. O2 content of the bath was calculated using a solubility of 205 µmol O2/ml solution when equilibrated with air. For the segments used in the separate determinations of lactate production, an O2 partial pressure of 0.95 atm was used. This was to ensure that these segments, which were mounted on glass rods and therefore perfused on only one side, would be adequately oxygenated. Tissues were generally given a test contraction induced by 80 mM KCl, relaxed by replacing this solution with PSS, then followed by the various test solutions. After complete washout of test substances, passive tension was quite reproducible. The difference between the values recorded before and after the initial 80 mM KCl stimulation expressed as a percent of the average passive tension was -1% ± 3% (n = 9). Lactate production was estimated from the lactate content of the bathing medium samples after a 30-minute incubation. As previously reported (Paul, 1983b), a substantial reduction in variance was obtained if the results during the test period were normalized to the basal value of the measured parameter. Control values before and after the test value were averaged to obtain the basal value used for this normalization. For determination of phosphorylase activities, the vessels were frozen in liquid N2 (see above) following a 30-minute incubation in the test solution. This duration was chosen for comparison to the lactate and O2 consumption measurements, which, along with isometric force, are in a steady state at this time. It has been reported recently that the activation of phosphorylase decreases with the duration of contraction following stimulation (Galvas et al., 1981; Silver and Stull, 1982; Paul, 1983a). Similar changes have been reported for Jo2 (Krisanda and Paul, 1983b).
1982). The data presented here are limited to assessing the role of phosphorylase in the regulation of steady state metabolic rates.

**Results**

**Oxygen Consumption**

Under isometric conditions, a reference contraction was obtained by adding KCl to the PSS. As previously reported (Paul, 1983b), maximal tension was found to occur in the range between 50 and 80 mM added KCl, and 80 mM was generally used. With a preload of approximately 20 mN/mm², the average active isometric force elicited was 38.0 ± 4.5 mN/mm² (n = 10), which agrees with that previously reported [42.9 mN/mm² (Paul et al., 1979; Paul, 1983b)]. It should be noted that, while these KCl-induced contractures are reproducible, they do not represent the maximum isometric force. Forces up to 2-fold greater can be elicited by histamine, which places the force-generating capacity of this preparation in the range observed for other vascular tissues (Paul, 1980). In order to test the relaxing effects of isoproterenol, a submaximal contraction was desirable. This was achieved by using added KCl in the range of 20–25 mM to achieve a near maximal contraction averaging 0.748 ± 0.064 (n = 11) of the maximum observed. Isoproterenol (10⁻⁶ M) was chosen again to produce near maximal effects. When it was added to the submaximal KCl contraction described above, the level of active isometric force was reduced to 0.094 ± 0.043 (n = 12) of that induced by KCl. The effects of these interventions on isometric force and oxygen consumption for a typical experiment are shown in Figure 2. The rate of oxygen consumption (J₀₂) with the maximum KCl contracture increases to approximately 1.6 times the basal rate, in agreement with previously reported values (Paul et al., 1979; Paul, 1983b). At submaximal contraction levels, the increased steady state J₀₂ was found to parallel closely the increase in active isometric force. Isoproterenol reduced both J₀₂ and ΔP₀ in a parallel fashion. Isoproterenol alone induced a

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Responses of porcine coronary artery oxygen consumption and isometric force to added KCl and isoproterenol. Upper record: output of polarographic electrode corresponding to bath O₂ concentration. The rate of O₂ consumption, calculated from the slope of these records is given below each measurement period. Lower record: isometric force measured simultaneously with tissue O₂ consumption. Arrows indicate the following changes in the bathing media: 80 mM KCl, KCl added to elevate bath concentrations by 80 mM (ΔP₀ = 34.3 mN/mm²); PSS, bathing solution replaced with fresh, oxygenated PSS (note rapid rise in polarographic output corresponding to increased bath P₀₂); 25 mM KCl, a submaximal isometric contracture (ΔP₀ = 30.8 mN/mm²) was elicited by increasing the bath KCl concentration by 25 mM; +10⁻⁶ M isoproterenol, indicates addition of this agonist.
small decrease in both J_o2 and ΔP_o, which was statistically significant only in the case of ΔP_o. As previously reported (Paul et al., 1979; Paul, 1983b), ouabain (10^-5 M) induces an increase in ΔP_o and a parallel increase in J_o2. Average data for these experiments are presented in Table 1.

**Lactate Production**

The effect of the various protocols on lactate production are also summarized in Table 1. As previously reported (Paul et al., 1979; Paul, 1983b), added KCl stimulates aerobic glycolysis. The increase in rate of lactate production (J_lac) at maximal stimulus levels compared to submaximal doses of KCl was proportionally larger than the increases observed in ΔP_o. Also as previously noted, the apparent rate determined in the initial 15- to 20-minute sample period was greater than found in subsequent periods. Isoproterenol (10^-8 M) alone had little effect on J_lac. However, when added to a submaximal KCl contracture, J_lac remained elevated in spite of a drop in both J_o2 and ΔP_o. As previously reported, 10^-5 M ouabain affected a drop in J_lac to about 50% below basal levels. J_lac thus could be increased or decreased independent of the direction of changes in J_o2 and ΔP_o.

**Phosphorylase**

To validate our techniques and protocols, we conducted a parallel series of experiments on rabbit aorta which allowed comparison with the well-controlled studies of Namm (1971). In the present work, the total specific activity of phosphorylase in rabbit aorta was 0.455 ± 0.036 (n = 47) µmol/(min-g wet weight), while the basal and maximal KCl-activated activity ratios were 0.117 ± 0.008 (n = 18) and 0.305 ± 0.032 (n = 7), respectively. These values agree remarkably well with the previously published (Namm, 1971), values of 0.443 ± 0.014 µmol/(min-g), 0.122 ± 0.011 and 0.300 ± 0.030, respectively. Thus, the results of our continuous enzyme-linked methods appear to agree with the results obtained by different methods. The total specific activity of porcine coronary artery phosphorylase, 0.102 ± 0.008 (n = 53) µmol/(min-g), was substantially lower than that measured for rabbit aorta. The reason for this difference is not known. However, the higher total phosphorylase activity of the rabbit aorta may be related to its higher metabolic rate (Paul, 1980, 1981). Using a Q10 of 2.5 to estimate the specific activity of 37°C and the observed activity ratios, it can be calculated that under basal conditions, glycogenolysis rate-limited by phosphorylase could account for 80% of the observed lactate production rate in porcine coronary artery. For rabbit aorta, on the other hand, J_lac similarly calculated on the basis of phosphorylase activity would be about 2-fold greater than the reported J_lac (Paul, 1983b). In both cases, the total phosphorylase activity is in considerable excess of that required to account for the maximal J_lac observed. As each glucose 1-phosphate produced from glycogen could account for six molecules of O2, as opposed to two lactate molecules, phosphorylase would not appear rate limiting for J_o2 under any condition.

### Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>80 mM KCl</th>
<th>25 mM KCl</th>
<th>25 mM KCl + 10^-6 M ISO</th>
<th>10^-6 M ISO</th>
<th>10^-5 M Ouabain*</th>
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<tbody>
<tr>
<td>J_o2</td>
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<td>0.007</td>
<td>0.082</td>
<td>0.004</td>
<td>0.074</td>
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<tr>
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<td>0.001</td>
<td>0.004</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>J_lac</td>
<td>56.9</td>
<td>5.1</td>
<td>50.7</td>
<td>7.6</td>
<td>59</td>
</tr>
<tr>
<td>SEM</td>
<td>39.9</td>
<td>4.7</td>
<td>25.2</td>
<td>5.5</td>
<td>22</td>
</tr>
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<td>10</td>
<td>12</td>
<td>22</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>ΔP_o</td>
<td>8.3</td>
<td>4.3</td>
<td>27.8</td>
<td>6.4</td>
<td>19</td>
</tr>
<tr>
<td>SEM</td>
<td>7.5</td>
<td>4.0</td>
<td>1.3</td>
<td>4.7</td>
<td>18</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>8</td>
<td>11</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Passive force</td>
<td>mN mm²</td>
<td>mN mm²</td>
<td>P(25)</td>
<td>P(25 + ISO)</td>
<td>P(ISO)</td>
</tr>
<tr>
<td>P_o</td>
<td>22.8</td>
<td>38.0</td>
<td>0.748</td>
<td>0.094</td>
<td>0.064</td>
</tr>
<tr>
<td>SEM</td>
<td>1.9</td>
<td>4.5</td>
<td>0.064</td>
<td>0.044</td>
<td>0.014</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

* Previously published, Paul et al., 1979.
† n = number of tissues.
on the phosphorylase activity ratio are presented in Table 2. The pattern of changes observed was the same for both porcine coronary artery and rabbit aorta. Added KCl increased the activity ratio to approximately the same degree as changes in ΔPc, ΔJo2, and, to a somewhat lesser extent, ΔJuc. Isoproterenol added to a submaximal KCl contracture resulted in an activity ratio that was elevated to an extent similar to that found with submaximal KCl alone. In the coronary artery, the addition of isoproterenol to a KCl contracture reduced Jo2 and ΔPc, while Juc remained elevated. In rabbit aorta, it should be noted that isoproterenol does not relax the contraction, and—though not studied in detail—appears not to alter Jo2 or Juc. Isoproterenol alone elicited only a small increase in phosphorylase activity which was not statistically significant. For rabbit aorta, this agreed with the findings of Namm (1971), who reported that isoproterenol increased the activity ratio of phosphorylase only at high concentrations which also elicited an increase in ΔPc. Ouabain also was found to increase phosphorylase activity, in parallel to its effect on the phosphorylase activity ratio are presented in Table 2, the changes are comparable to those observed in Jo2 and Juc (Table 1). The basal activity ratio of porcine coronary artery was higher than that of rabbit aorta. The basis for this difference is unknown, but, as similar techniques were used, it is unlikely to be artifactual.

The magnitude of the observed changes in activity ratio were considerably smaller in the porcine coronary artery than in rabbit aorta. The changes from the basal value, however, were statistically significant in all cases, with the exception of isoproterenol, as previously noted, and for ouabain, which had a P < 0.1. Although the absolute change in phosphorylase activity in coronary artery is small, when expressed in terms of the percent change from basal values (Table 2), the changes are comparable to those observed in Jo2 and Juc (Table 1). The basal activity ratio of porcine coronary artery was higher than that of rabbit aorta. The basis for this difference is unknown, but, as similar techniques were used, it is unlikely to be artifactual.

### Table 2

<table>
<thead>
<tr>
<th>Condition</th>
<th>Basal</th>
<th>80 mM KCl</th>
<th>25 mM KCl</th>
<th>25 mM KCl +10^-6 M ISO</th>
<th>ISO 10^-6 M Ouabain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit aorta</td>
<td>Mean</td>
<td>0.455</td>
<td>0.117</td>
<td>0.305</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.036</td>
<td>0.008</td>
<td>0.032</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>n *</td>
<td>47</td>
<td>18</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Porcine coronary artery</td>
<td>Mean</td>
<td>0.102</td>
<td>0.171</td>
<td>0.268</td>
<td>0.217</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.008</td>
<td>0.014</td>
<td>0.024</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>53</td>
<td>14</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

| % Change from basal | 161.1 | 79.8 | 92.6 | 32.7 | 67.0 |
| % Change from basal | 56.7 | 26.9 | 45.0 | 21.6 | 32.8 |

* n = number of tissues.

**Discussion**

It is generally assumed that, in the absence of uncoupling, increases in metabolic rates reflect increased ATP utilization by various cellular processes. There is a substantial amount of evidence indicating a strong correlation between oxidative metabolism and isometric force, presumably reflecting actomyosin ATPase. There has been far less evidence correlating function with aerobic glycolysis, although, under certain conditions, it also appears to be related to force (Peterson and Paul, 1974). The substantial amount of aerobic glycolysis observed in vascular tissue has aroused considerable speculation, as this is observed in relatively few cell types, such as ascites tumor cells and retinal cells (Krebs, 1972). Recently, we have shown that conditions known to alter Na-K transport also effect lactate production (Paul et al., 1979; Paul, 1983b). In the present study, additional evidence is presented indicating that oxidative and glycolytic metabolism are separable. Isoproterenol, in the presence of added KCl, lowers oxidative metabolism and ΔPc while stimulating lactate production. This is the converse of the effects observed in the presence of ouabain, which elicits increases in Jo2 and ΔPc, but inhibits Juc. As ouabain is known to inhibit Na-K transport and there is evidence (Scheid et al., 1979) that isoproterenol stimulates Na-K transport, these results are consistent with the hypothesis that aerobic glycolysis is related to the energetics of Na-K transport in VSM. The mechanisms underlying this functional compartmentalization are as yet unclear.

Our studies on phosphorylase, a key enzyme in the glycogenolytic cascade, were undertaken to determine its role, if any, in the observed independent behavior of oxidative phosphorylation and aerobic glycolysis. The specific activities of phosphorylase compared with the overall metabolic rates observed suggest that it could play a rate-limiting role for aerobic glycolysis.
particularly for porcine coronary artery. Under stimulation of added KCl, Jo, Jiac and the phosphorylase activity increase as does isometric force. There is evidence (Anderson, 1976) that Na-K transport also increases. Addition of isoproterenol to a KCl-induced contraction with porcine coronary artery results in a relaxation of ΔP\textsubscript{in} and J\textsubscript{0}, but not J\textsubscript{iac}, which remains above the basal J\textsubscript{iac}. This evidence could indicate a strong coupling between phosphorylase and J\textsubscript{iac} and, because Na-K transport is likely to be increased under these conditions, between phosphorylase and Na-K transport. However, ouabain—which increases ΔP\textsubscript{in} and J\textsubscript{0}, in a parallel fashion—does not inhibit phosphorylase, whereas J\textsubscript{iac} is strongly inhibited. This is not consistent with a rate-limiting role for phosphorylase in respect to aerobic lactate production, are supported by recent results which indicate that exogenous glucose is the substrate for the observed lactate production (Lynch and Paul, 1983).

The activity of phosphorylase may instead simply be related to the level of intracellular Ca\textsuperscript{2+} concentration. Namm’s (1971) results for rabbit aorta indicated that phosphorylase activity increased under a variety of conditions that increased ΔP\textsubscript{in} and, presumably, intracellular Ca\textsuperscript{2+}. It was further shown in this study that activation of phosphorylase required the presence of external Ca\textsuperscript{2+}, whereas isoproterenol alone at concentrations not inducing contraction had no effect. Our results were similar, although there was a tendency to exhibit a slight increase in the presence of isoproterenol alone, which, however, was not statistically significant. If one makes the assumption that Ca\textsuperscript{2+} is the stimulus for phosphorylase activation, the increase in phosphorylase and ΔP\textsubscript{in} in the presence of ouabain would be consistent with Namm’s hypothesis. J\textsubscript{0}, again follows the increase in ΔP\textsubscript{in}, presumably reflecting the increase in actomyosin ATP\textsubscript{ase}. The control mechanism for the inhibition of J\textsubscript{iac} correlated with the decrease in Na-K transport concomitant with an increased J\textsubscript{0} is not known; however, some form of enzymatic compartmentation would appear to be implicated by these results.

The observation that isoproterenol can relax a KCl-induced contracture while phosphorylase activity is increased decrease in Na-K transport concomitant with aerobic lactate production, are supported by recent results which indicate that exogenous glucose is the substrate for the observed lactate production (Lynch and Paul, 1983).

The activity of phosphorylase may instead simply be related to the level of intracellular Ca\textsuperscript{2+} concentration. Namm’s (1971) results for rabbit aorta indicated that phosphorylase activity increased under a variety of conditions that increased ΔP\textsubscript{in} and, presumably, intracellular Ca\textsuperscript{2+}. It was further shown in this study that activation of phosphorylase required the presence of external Ca\textsuperscript{2+}, whereas isoproterenol alone at concentrations not inducing contraction had no effect. Our results were similar, although there was a tendency to exhibit a slight increase in the presence of isoproterenol alone, which, however, was not statistically significant. If one makes the assumption that Ca\textsuperscript{2+} is the stimulus for phosphorylase activation, the increase in phosphorylase and ΔP\textsubscript{in} in the presence of ouabain would be consistent with Namm’s hypothesis. J\textsubscript{0}, again follows the increase in ΔP\textsubscript{in}, presumably reflecting the increase in actomyosin ATP\textsubscript{ase}. The control mechanism for the inhibition of J\textsubscript{iac} correlated with the decrease in Na-K transport concomitant with an increased J\textsubscript{0} is not known; however, some form of enzymatic compartmentation would appear to be implicated by these results.

The observation that isoproterenol can relax a KCl-induced contracture while phosphorylase activity is maximally elevated may provide some insight to the mechanism(s) underlying β-adrenergic relaxation in coronary vessels. Many hypotheses for β-adrenergic relaxation have been put forth. See, for example the recent review of Kurikiyama et al. (1982) from which two major classes can be distinguished: (1) mechanisms involving the lowering of cytoplasmic free Ca\textsuperscript{2+}, for example, by increased Ca\textsuperscript{2+} uptake by microsomal vesicles (Mueller and van Breeman, 1977) or increased Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange (Scheid et al., 1979); (2) mechanisms involving a desensitization of the contractile regulatory system to Ca\textsuperscript{2+}, for example, deactivation of myosin light chain kinase via phosphorylation mediated by cAMP-dependent protein kinase (Adelstein and Hathaway, 1979; Silver and DiSalvo, 1979). Although these mechanisms are not necessarily mutually exclusive, our data support the latter class of mechanism.

Activation of phosphorylase a is dependent on Ca\textsuperscript{2+} mediated by the absolute requirement of phosphorylase kinase for Ca\textsuperscript{2+} (Cohen et al., 1980). Actomyosin interaction in smooth muscle also depends on Ca\textsuperscript{2+}, mediated, at least in part, by a similar enzyme cascade involving myosin light chain kinase, a calcium-dependent enzyme (Hartshorne and Siemannowski, 1981). In the presence of calmodulin, the apparent Ca\textsuperscript{2+} sensitivity for isometric force in "chemically skinned" vascular smooth muscle was recently characterized with a K\textsubscript{m} of 10\textsuperscript{-7} M (Ruegg and Paul, 1982). This value, which is lower than earlier reports (Filó et al., 1965), is similar to that reported for the most Ca\textsuperscript{2+}-sensitive, phosphorylated form of phosphorylase kinase (Cohen et al., 1980). Thus, one would anticipate that the maximal activation of phosphorylase a observed in the presence of isoproterenol and KCl would be indicative of an intracellular Ca\textsuperscript{2+} concentration sufficient for actin-myosin interaction. The relaxation of force observed under these conditions thus suggests that the calcium sensitivity for actin-myosin interaction may be decreased. This is consistent with the hypothesis that β-adrenergic relaxation is mediated through a phosphorylation-induced decrease in Ca\textsuperscript{2+} sensitivity of myosin light chain kinase. The conclusion rests on the assumption that the Ca\textsuperscript{2+} sensitivities observed in studies on isolated proteins can be extrapolated to the in vivo condition. Whereas this is not unreasonable, these results could simply indicate differences in in vivo Ca\textsuperscript{2+} sensitivities. However, the applicability of this mechanism has been verified in both actomyosin isolated from vascular smooth muscle (Silver et al., 1981) and in "chemically skinned" vascular smooth muscle (Kerrick and Hoar, 1981; Ruegg and Paul, 1982). Our results with living VSM suggest that this mechanism may also play a major role in vivo.

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R J Paul

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