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OXYGEN CONSUMPTION RATE IN VASCULAR SMOOTH MUSCLE: RELATION TO ISOMETRIC TENSION

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SUMMARY

Advances in muscle energetics require knowledge of the rates of the driving chemical reaction. Vascular smooth muscle, 2–3 orders of magnitude slower than amphibian skeletal muscle, permits continuous measurement of reaction rates. Oxygen consumption rates in the steady state appear to be a valid measure of the rate of the driving chemical reaction. The relation between isometric force and oxygen consumption rate is linear, and thus consistent with nonequilibrium thermodynamic analysis.

The understanding of the mechanochemical nature of muscle contraction has progressed slowly since the initial demonstration that ATP is split in the fundamental mechanochemical event[1]. To determine the mechanochemistry of any system requires a detailed knowledge of not only the net amount of chemical breakdown, but also the rates of chemical reaction under various mechanical constraints[2]. Because of the extremely rapid contraction and intrinsic variability in amphibian skeletal muscle, these parameters are practically impossible to measure. In particular, the rate of reaction during unloaded contraction, an important criterion necessary to distinguish between current theories of muscle energetics[3–5], has proved impossible to measure with the necessary resolution. Furthermore, the estimation of rates of reaction based on measurements of heat production has been shown to be invalid on theoretical grounds[6], and important experimental discrepancies remain unresolved[7].

Our investigation has been directed towards finding a muscle system suitable for these mechanochemical measurements. Using maximum velocity as an index of muscular speed, vascular smooth muscle is 2–3 orders of magnitude slower than frog sartorius[8]. For this reason, vascular smooth muscle was chosen as a system in which continuous measurements of the driving chemical reaction could be made. For this tissue, it is known that ATP is split during contraction and, unlike amphibian skeletal muscle, there are no large pools of high energy phosphates[9,10]. Furthermore, it has been estimated that vascular smooth muscle consumes 3 times the amount of energy stored in its preformed high energy phosphate compounds in generating maximum isometric tension[11]; thus, ATP must be synthesized continuously during activity. For these reasons, the monitoring of intermediary metabolism in these

tissues can be expected to be an adequate measure of the ATP production. The contribution of aerobic glycolysis to the total ATP production is small*. Although it has been observed that oxygen consumption rates in vascular smooth muscle increase during contraction, in previous experiments continuous rate measurements and a well characterized mechanical state were not achieved[14]. Our experiments were designed to measure the input of chemical energy by monitoring continuously the oxygen consumption rate, while simultaneously following the generation of isometric force.

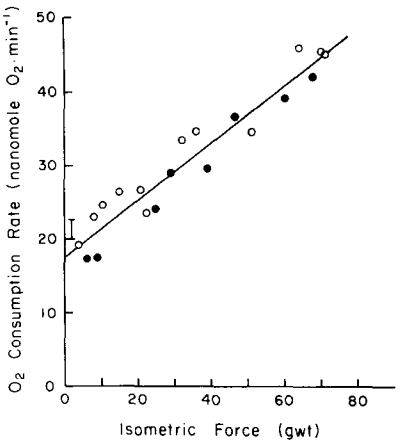

Fig. 1. Relationship between oxygen consumption rate and isometric force for Tissue 3b of Table I. ○, measurements taken with increasing epinephrine concentration; ●, measurements taken with decreasing epinephrine concentration; bar, basal oxygen consumption rate prior to stimulation. Isometric force is expressed in gram-weight (1 gwt = 980 dynes).

As with other muscle preparations, isometric force at maximum stimulation can be varied by changing the initial length of the tissue. In addition, at fixed muscle length, a graded force response in bovine mesenteric vein can be produced by varying the concentration of catecholamine in the bathing solution. A linear relation has been observed (Fig. 1) between this graded isometric force and the oxygen consumption rate in the steady state. The average proportionality factor (for 23 tissues) between the rate of oxygen consumption and isometric force during periods of both increasing and decreasing epinephrine concentration in the bath is 0.193 ± 0.011 nmole $O_2 \cdot min^{-1} \cdot g^{-1}$ dry weight $\cdot (gwt \cdot cm^{-2})^{-1}$. Although this factor differs for individual tissues, the linear relationship is reproducible and is maintained through several varied cycles of stimulation and relaxation.

A linear relation between generated force and the chemical turnover rate of the driving chemical reaction is consistent with nonequilibrium thermodynamic analysis of muscle contraction[15]. In order to be able to interpret the linear relation between oxygen consumption rates and force in this way, one must first determine

* Under aerobic conditions, in bovine inferior vena cava, 57% of the glucose is metabolized *via* glycolysis and 43% by oxidation[12]. Oxidative metabolism, even under these conditions, accounts for 93% of the total ATP production. Mesenteric vein, being a thinner preparation, would be expected to have even less oxygen-diffusion-limited stimulus of aerobic glycolysis[13].

(a) whether oxygen consumption rates truly reflect the energetic requirements of the contraction and (b) whether this correlation signifies a relation of state between force and the rate of oxygen consumption. Further experiments were undertaken to clarify these points.

In the absence of glucose as substrate, under conditions of maximum epinephrine stimulation, after less than 2 h the tissue cannot maintain active (total *minus* passive) tension. Oxygen consumption rates recorded during this period of declining tension were found to fall linearly with force (4 experiments, correlation coefficients range from 0.85 to 0.92). Initial values of oxygen consumption rate and isometric force could be restored by the addition of glucose or other substrates of oxidative metabolism. However, though it is clear that the oxygen consumption rate is related to the ability of the tissue to generate and maintain tension, it may be suggested that the rate of oxygen consumption is more strongly related to the dosage of epinephrine.

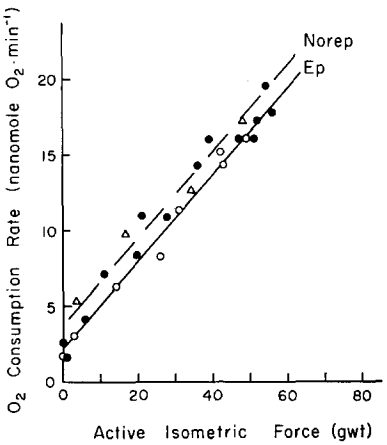

Fig. 2. Increase in oxygen consumption rate (stimulated *minus* basal) plotted against active isometric force (total force *minus* passive force at L_0) for Tissue 11a of Table I under three independent experimental conditions. △, measurements made with varying isometric length at fixed epinephrine concentration; ○, measurements made with varying epinephrine concentration at fixed length; ●, measurements made with varying norepinephrine concentration at fixed length. All measurements were made on the same vein loop.

The following evidence suggests that this is not the case. If one maintains maximum pharmacological stimulation and allows the tissue to contract to its minimum contracted length (L_{min}) at which no active tension is generated, our experiments indicate that the oxygen consumption returns to near basal levels. The oxygen consumption rate at L_{min} differs from the basal oxygen consumption rate by $+9.6 \pm 1.9\%$ for 9 experiments. Preliminary studies also show that the oxygen consumption rate-isometric force linear relation is maintained when varying the isometric force by varying the initial length of the tissue under conditions of maximum stimulation (Fig. 2). These results appear to be independent of the stimulant used. A detailed study using an alternative stimulant (norepinephrine) indicates that the

TABLE I

SLOPE OF OXYGEN CONSUMPTION RATE *VERSUS* ISOMETRIC FORCE LINE GENERATED AT L_0 IN BOVINE MESENTERIC VEIN

Tissues 11a–13 are the 5 paired stimulus experiments. For any single tissue, the difference between the oxygen consumption rate–isometric force slope when stimulating with epinephrine and the slope when stimulating with norepinephrine is not statistically significant at the 5% confidence level. The *significance stated with slope averages is the probability* that the given slope arose through random sampling error in a population with true zero slope. Lettered tissue numbers indicate experiments done on different sections of a single vein. Slope is expressed in units of nmole $O_2 \cdot min^{-1} \cdot g^{-1}$ dry wt $\cdot (gwt \cdot cm^{-2})^{-1}$. Isometric force per unit area calculated by $P_0 \cdot L_0 \cdot g^{-1}$ dry weight. L_0 reference length was chosen as that length at which a stable passive force of 1 gwt was maintained. r = product-moment coefficient of correlation. N = number of points per experiment used in determining least squares fit line.

Tissue No.	*Slope (stimulus: epinephrine)*	r	N	*Tissue No.*	*Slope (stimulus: epinephrine)*	r	N	*Tissue No.*	*Slope (stimulus: norepinephrine)*	r	N	*Difference in slope between the two stimuli in the same vein*
1a	0.320 ± 0.028	0.99	7	7	0.206 ± 0.027	0.85	24					
1b	0.115 ± 0.024	0.96	7	8	0.227 ± 0.023	0.97	8					
1c	0.170 ± 0.016	0.96	11	9a	0.259 ± 0.023	0.98	8					
1d	0.121 ± 0.015	0.95	9	9b	0.268 ± 0.015	0.99	8					
2a	0.123 ± 0.016	0.90	24	10a	0.221 ± 0.016	0.99	9	14	0.201 ± 0.016	0.99	6	
2b	0.154 ± 0.030	0.83	16	10b	0.218 ± 0.014	0.99	8	15	0.297 ± 0.040	0.95	9	
3a	0.282 ± 0.021	0.95	20	11a	0.162 ± 0.017	0.97	8	11a	0.161 ± 0.020	0.98	14	0.001 ± 0.026
3b	0.167 ± 0.015	0.94	19	11b	0.185 ± 0.014	0.94	26	11b	0.215 ± 0.011	0.96	29	-0.030 ± 0.018
4	0.171 ± 0.046	0.90	5	12a	0.189 ± 0.022	0.95	10	12a	0.256 ± 0.022	0.97	10	-0.067 ± 0.032
5a	0.127 ± 0.026	0.79	9	12b	0.224 ± 0.022	0.98	6	12b	0.188 ± 0.024	0.91	8	0.036 ± 0.031
5b	0.212 ± 0.063	0.77	19	13	0.270 ± 0.067	0.94	6	13	0.367 ± 0.054	0.89	8	-0.097 ± 0.088
6	0.192 ± 0.018	0.93	23									
Variance weighted mean and standard error (all experiments in each stimulus class):					0.193 ± 0.011				0.212 ± 0.015			-0.020 ± 0.023
Number of determinations:					$N = 23$				$N = 7$			$N = 5$
Significance of slope:					$P < 0.001$				$P < 0.001$			
Significance of difference of mean values:					Difference between means $= -0.019 \pm 0.017$ ($df = 28$, $t = 1.15$); not significant at the 5% confidence level; probability of random occurrence = 26%							
Significance of mean value of differences in paired stimulus experiments (5 pairs):					Weighted mean difference $= -0.020 \pm 0.023$ ($df = 3$, $t = 0.86$); not significant at the 5% confidence level; probability of random occurrence = 47%							

TABLE II

BASAL OXYGEN CONSUMPTION RATE AND INTERCEPTS AT ZERO ACTIVE FORCE OF OXYGEN CONSUMPTION RATE *VERSUS* ISOMETRIC FORCE LINES

Basal oxygen consumption rate was measured during the final 30 min of the 120-min initial equilibration period in the muscle chamber. Values given are the variance weighted means and standard errors from all experiments in each stimulus class. The significance stated is the probability that the given difference arose from random sampling error alone. Mean basal ($N=30$) is 0.488 ± 0.025 μmole $O_2 \cdot min^{-1} \cdot g^{-1}$ dry weight.

Stimulus	*Number of experiments*	*Intercept − basal*	$\frac{\textit{Intercept} - \textit{basal}}{\textit{Basal}}$ (%)
		$[1/N]\Sigma(I_i - B_i)$	$[1/N]\Sigma(I_i - B_i)/B_i$
Epinephrine	20	0.0154 ± 0.0060	2.5 ± 1.0
Norepinephrine	7	0.0150 ± 0.0038	1.9 ± 0.9
Difference (epinephrine − norepinephrine):		0.0004 ± 0.0078	0.6 ± 1.4
Significance of difference:		$P=0.95$	$P=0.65$

oxygen consumption rate-graded isometric force correlation is maintained (Fig. 2). The difference in slopes measured using the two methods of pharmacological stimulation is less than 10%. This difference is not statistically significant when tested either by comparing the means of all experiments or by a more restrictive pairwise comparison on individual tissues (Table I). Furthermore, it is observed that the intercept at zero active force of the oxygen consumption rate-isometric force line (least squares fit), which is the same when generated with either epinephrine or norepinephrine, differs from the basal oxygen consumption rate prior to stimulation by less than 3% (Table II). Preliminary results using a non-adrenergic stimulant (histamine) are consistent with the stimulus non-specificity of the above results. In contrast to the linear relation between the oxygen consumption rate and isometric force, the relationship between oxygen consumption rate and epinephrine dosage is strikingly non-linear (oxygen consumption rate and epinephrine concentration are related exponentially).

The above findings strongly suggest that in the steady state the two pharmacological agents used do not stimulate substantially any metabolism not directly related to the generation of force. Recent studies of the effect of norepinephrine on cardiac muscle have yielded similar conclusions for that tissue[16]. Our results indicate that the rate of oxygen consumption is a valid index of contractile energetics in these tissues. The linear relation between isometric tension and chemical input, as observed by the direct measurement of rates, is consistent with a nonequilibrium thermodynamic analysis of muscle contraction.

EXPERIMENTAL

Mesenteric veins were excised from 6–8-week-old calves within 30 min of exsanguination, stripped of adventitia, and immersed in iced saline. The saline contained: 118 mM NaCl, 5.32 mM KCl, 1.54 mM NaH_2PO_4, 1.19 mM $MgSO_4$, 24.9 mM

$NaHCO_3$ and 2.53 mM $CaCl_2$; the pH was 7.4 when gassed with air–CO_2 (95:5, v/v) at 37 °C. The vein was cut longitudinally and unfolded such that a strip 1 cm × 4 cm was formed. The ends of the strip were sewn together forming a longitudinal loop of 1 cm × 2 cm, approx. 40 mg dry weight and cross section area 0.1 cm^2. After overnight storage at 0 °C, the vein loop was mounted in a glass and stainless steel, well-stirred chamber (20-ml volume). The chamber was equipped with a Clark type polarographic oxygen electrode and a mounting assembly for the vein loop capable of measuring force and length. Glucose (10 mM) was introduced and basal oxygen consumption was recorded for the next 2 h. Upon maximal stimulation with epinephrine (3 μg/ml bath contents), isometric tension increased to typically 50 gwt ($t_{\frac{1}{2}} = 5$ min), while oxygen consumption rate rose immediately to approximately twice basal. This increased level of both isometric tension and oxygen consumption rate could be maintained for several hours without significant fade. The pharmacological stimulants added were then diluted at 15–30-min intervals by flushing the chamber with pre-determined volumes of fresh saline and glucose. Upon such dilutions, oxygen consumption rate and isometric tension fell in parallel to new stable submaximal values, which again could be maintained indefinitely in the absence of further perturbations. Sequential dilutions were continued until the tissue returned to passive tension and basal oxygen consumption rate. The stimulation cycle could be reversed by sequential additions of submaximal doses of stimulant. Though no effect on either oxygen consumption rate or isometric tension was observed as a function of oxygen tension, it was maintained to $\pm 40\%$ of air by flushing the chamber thoroughly at 45–60 min intervals. The oxygen consumption rate was normalized to tissue dry weight, and the isometric force to cross section area (using $L_0^{-1} \cdot$ dry weight to approximate the area) to account for possible variations in muscle content of the tissue.

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