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Cross Bridge ATP Utilization in Arterial Smooth Muscle

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Summary. Measurements of oxygen consumption and lactate production were used to estimate the ATP production in porcine carotid arteries. Changes in the total energy metabolism were correlated with changes in force which was altered by changing the muscle length under maximum stimulation. This tension-dependent metabolism could be used to distinguish between cross bridge and other ATP utilization. The tension-dependent metabolism was equal under K^+-depolarization or histamine stimulation. Tension cost was 300x smaller than skeletal muscle while the cross bridge cycle duration 30x longer.

Key words: Arterial muscle metabolism – Actomyosin ATPase – Mechanochemistry – Oxygen Consumption.

Our understanding of the mechanochemistry of arterial smooth muscle is limited by the lack of knowledge of the cross bridge ATPase *in situ*. It is currently impossible to distinguish between the chemical energy required by actomyosin interactions from that of other processes accompanying stimulation. This problem is exacerbated in smooth muscle in which unambiguous "relaxed" conditions are often difficult to obtain and the rates of metabolism in stimulated muscles are seldom more than twice the pre-stimulus levels. The magnitude of the measured metabolic changes accompanying contraction are therefore critically dependent on the assumption of a meaningful and unperturbed baseline. It is not surprising that under the various modes of stimulation these "increases" in metabolism vary considerably [7]. Conclusions from a variable mechanicochemical coupling to the existence of a "catch" mechanism are compatible with the available evidence [10].

To isolate the true actomyosin ATPase *in situ*, we have estimated the total cellular ATP hydrolysis rate in a fully stimulated muscle at various lengths. The interpretation of the force-length relations found in smooth muscle in terms of a sliding filament model is supported by a growing body of evidence [3]. Thus, changes in the total rate of ATP utilization with isometric force at various muscle lengths can be most simply attributed to changes solely in the number of actomyosin interaction sites. The tension-dependent metabolism under these conditions can be viewed in terms of cross bridge ATP utilization. This interpretation is independent of previous "basal" states and requires only that the ATP utilization of the new metabolic state under maximal stimulation (from which the actomyosin ATPase is effectively subtracted by changing the muscle length) is steady.

Although direct measurement of ATP hydrolysis is possible in arteries [2], ongoing metabolic resynthesis of 0.5 to 2 μmole ATP·(min·g)$^{-1}$ [13] obscures any interpretation. As the total high-energy phosphate (ATP + PCr) content of arterial muscle is less than 2 μmole·g^{-1} [10], the available supply of chemical energy is not sufficient to maintain an isometric contraction. Interpretation of the ATP hydrolysis in muscles poisoned to inhibit ATP resynthesis [5], is unclear in view of the rapidly diminishing energy reserves. Hence we have measured the oxygen consumption and aerobic lactate production to estimate the ATP hydrolysis in terms of its resynthesis by intermediary metabolism. ATP utilization and production are equal in the steady state; measurements herein reported were made only after steady levels of both isometric force and oxygen consumption rate were obtained.

Methods

The experiments were performed on media strips of porcine carotid artery, in which the smooth muscle cells show a uniform, nearly circumferential orientation [6]. This allows these measurements made on circumferential strips to be simply interpreted in terms of length changes imposed in the axial direction of the smooth muscle cells. Measurements were made as previously described for venous muscle [12, 14] in a chamber modified to the size requirements of this artery. Preparation of the arterial media strips was similar to that described by Herlihy and Murphy [6], with the exception that a larger strip width (1.5 cm) was used to increase the resolution of the metabolic measurements. A physiological salt solution with 10 mM Glucose [12, 14] was equilibrated with a gas containing 40% O_2 to insure that diffusion of O_2 would not be rate limiting [16]. The polarographic method allowed direct determination of the rate of O_2 consumption (J_{O_2}). Lactate production was determined using an enzymatic technique on samples of the bathing medium [15]. The chemical resolution required sample durations of the order of 30 min., the rate of lactate production (J_{lac}) is thus an average over the interval and not of the same order of precision as J_{O_2}. The steady state rate of ATP utilization (J_{ATP}) is calculated from J_{O_2} and J_{lac} using the stoichiometry of standard biochemical pathways, the error bounds of which have been previously discussed [15]. Stimulation of the muscle was achieved by either K^+-depolarization, using a high K^+ saline in which an equimolar substitution of K^+ for Na^+ was made, or pharmacologically, using histamine chloride at 0.1 mM.

Results

The relevant data are summarized in Table 1 in terms of the parameters from the linear regression of the total metabolic rate on active isometric force (Po). From such analysis one obtains the tension-independent and tension-dependent (*i.e.* actomyosin dependent) components of the total metabolism. An example of such a regression is given in Fig. 1, showing J_{O_2} *vs.* P_o. The scatter in the data is typical of similar experiments in skeletal muscles in which the data structure is composed of one point per animal [18]. As the methods used in this study were non-tissue destructive, results on single arteries indicate that this scatter is primarily due to animal variability; predominantly occuring in the absolute levels of metabolism. High pre-stimulus rates were associated with high rates of total metabolism under stimulated conditions at both the optimal length for force development (L_{opt}) and at the fully contracted length at which no active force was developed (L_{min}); an example is shown by the symbols in squares in Fig. 1. The tension-dependent component thus showed less variability, as can be more clearly seen with measurements on the same tissue. The dependence of J_{O_2} on force alone can be estimated as the paired difference in a single artery: $[J_{O_2}(L_{opt}) - J_{O_2}(L_{min})] \cdot P_o^{-1}$. This paired variate in 8 arteries was 0.071 ± 0.014 μmole·(min·g)$^{-1}$ per Kgwt·cm^{-2} (± SEM, $P < 0.002$) which is not statistically different from the same parameter estimated from the regression slope over all tissues.

The amount of aerobic glycolysis was significantly different under the different stimuli. Under K^+-depolarization, J_{lac} tended to be lower than previous "basal" levels; however, J_{lac} was still correlated with force development. Under histamine stimulation, J_{lac} was significantly increased as is reflected most strongly in the intercepts. Though comparable in molar rates, aerobic glycolysis accounts for only 15% to 25% of the total ATP production. Significantly, the tension-dependent metabolism in terms of ATP utilization was equal, averaging 0.64 μmole ATP·(min·g)$^{-1}$ per Kgwt·cm^{-2} under either stimulus.

Table 1. Tension-dependent and tension-independent components of aerobic metabolism obtained from the linear regressions of the total rates of oxygen consumption, lactate production and the calculated ATP utilization against isometric force at varying muscle length under supermaximal stimulation in porcine carotid artery. $J_X = B_1 + B_2 \cdot P_o$.

J_X	Stimulus	B_1 Tension Independent $\frac{\text{umole}}{\text{min·g}}$	B_2 Tension Dependent $\frac{\text{umole/(min·g)}}{\text{Kgwt/cm}^2}$	N*	R**
J_{O_2}	Hist	.073	.083	27	.73
J_{O_2}	K^+	.090	.093	23	.55
J_{lac}	Hist	.159	.089	25	.44
J_{lac}	K^+	.073	.072	22	.42
J_{ATP}†	Hist	.715	.626	25	.70
J_{ATP}	K^+	.689	.655	21	.59

* Number of points used in least square linear regression; from 18 arteries under histamine stimulation, and 16 arteries studied under K^+-depolarization.

** Pearson product-moment coefficient of correlation.

† Calculated as $6.42 \times J_{O_2} + 1.25 \times J_{lac}$ for individual points.

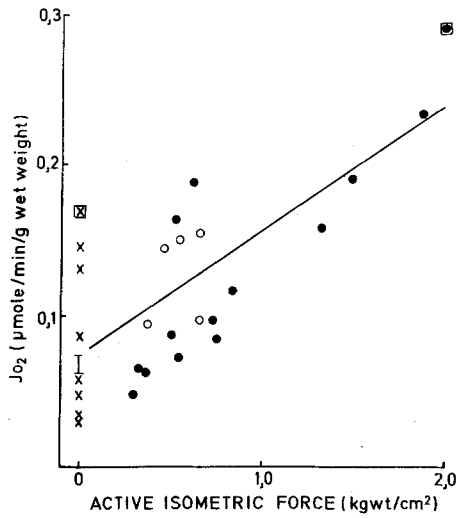

Fig. 1. Relation between the rate of oxygen consumption and active isometric force generated by varying tissue length while under maximal histamine stimulation. Measurements made at three reference lengths: ●'s, L_{opt}, set to maintain a pre-stimulus tension of 160 gwt/cm²; O's, L_o, set to zero pre-stimulus tension; X's, L_{min}, maximum contracted length under stimulation at which no active tension was generated. Vertical bar shows mean pre-stimulus O_2 consumption rate (± SEM, N = 31). Points in squares are an example of measurements on a single tissue. Regression parameters are given in the first row of Table 1.

Discussion

At the maximum force observed of 2 Kgwt·cm^{-2}, 1.3 µmole ATP·(min·g)$^{-1}$ of a total metabolic rate of 2 is tension dependent. This value for the *in situ* actomyosin ATPase at 37 °C may be compared to other biochemical estimates using a Q_{10} of 2.5. Using the specific actomyosin ATPase of 17 nmole·(min·mg)$^{-1}$ at 21 °C [8], 1.6 nmole myosin per mg actomyosin [19] and 16 nmole myosin per gram arterial media [9], a value of 0.73 mole ATP·(min·g)$^{-1}$ can be calculated for the whole media. This and the value of 1.5 obtained from the ATPase of arterial homogenates [8] are in reasonable agreement with the value obtained in the living artery.

The tension cost, *i.e.*, the ATP hydrolysis rate per unit isometric force, is 10 times smaller than the steady state value of frog sartorius at 0 °C [11], and nearly 300 times less when compared at the same temperature. The low tension cost of arterial muscle is striking when compared to the most economical tonic contraction in the lamellibranch catch muscles. The tension-dependent O_2 consumption measured by Baguet and Gillis [1] in ABRM at 20 °C is 10 fold smaller than in arteries at 37 °C, and would differ by only a factor of 2 when compared at the same temperature. Though highly economical, the tension dependence of the arterial metabolism argues against a special "locking" mechanism for tension maintenance without concommitant ATP splitting.

It is of interest to compare the relative tension cost to the average cross bridge cycle duration. Assuming one cross bridge per myosin and full participation, this duration can be estimated by the myosin content divided by the rate of ATP hydrolysis. The arterial cycle duration of 0.75 sec. is not substantially different from 0.33 to 0.7 sec. calculated for frog sartorius at 0 °C [4, 11]; comparison at the same temperature would lengthen the arterial cycle by a factor of 30. Thus one has a disparity of 5 to 10 fold between cycle duration and tension cost relative to skeletal muscle. This suggests that factors in addition to the low actomyosin ATPase, as for example a longer effective sarcomere length relative to skeletal muscle [17], may be involved in the low tension cost of arterial smooth muscle.

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