Activation of Contraction and ATPase Activity in Intact and Chemically Skinned Smooth Muscle of Rat Portal Vein

Dependence on Ca++ and Muscle Length

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SUMMARY. The mechanical manifestations of muscle contraction (force development or shortening) are accompanied by an increased turnover of chemical energy (ATPase activity, $J_{ATP}$). In intact rat portal veins activated by high potassium medium to produce graded contractions at different levels of extracellular calcium, a linear dependence of oxygen consumption on force was found. The slope of the relation (metabolic tension cost) was higher during early stages than during later stages of contraction, possibly reflecting a transient high crossbridge ATP turnover rate. Chemically skinned (Triton X-100) rat portal vein preparations were used to study the energy turnover of the smooth muscle contractile system under constant activation. In these preparations, $J_{ATP}$ increased on activation by calcium in the presence of 1 $\mu$M calmodulin, remained constant for maintained contractures, and decreased promptly on relaxation. Force declined with each repeated contraction at optimal calcium level (10$^{-8}$ M), but the relation between force and $J_{ATP}$ remained invariant and agreed with that of the intact muscle during the transient of high ATP turnover. Calcium activation in the range 10$^{-9}$ to 10$^{-4}$ M caused a progressively steeper (nonlinear) increase in $J_{ATP}$ with force. Length-force relations showed a lower relative force at muscle lengths below $L_m$ in the intact than in the skinned tissue, possibly indicating depression of excitation-contraction coupling. The slope of the relation between $J_{ATP}$ and force was lower when length was varied than when calcium was varied. At a length where no external force was produced, the activated muscle had a significantly higher $J_{ATP}$ than while relaxed (10$^{-9}$ M Ca++). Together with previous results showing calcium dependence of the force-velocity relation, the present study indicates an influence of calcium on crossbridge kinetics in smooth muscle. (Circ Res 53: 695-702, 1983)

THE relation between force and ATPase activity in smooth muscle is influenced by several factors, some of which may relate to the activation process and some, possibly, to characteristics of the actin-myosin interaction (cf review by Paul, 1980). During development of isometric force in K$^+$ contractures in rat portal vein and swine carotid artery, there is a period of transiently increased energy turnover (Arner and Hellstrand, 1980; Hellstrand and Paul, 1983; Paul et al., 1983). These studies correlate with observations of transient variations in the maximal shortening velocity (Uvelius and Hellstrand, 1980; Dillon et al., 1981). Mechanical and metabolic findings may reflect a potential for regulation of the kinetics of crossbridge interaction which may be unique for smooth muscle. The development of preparations devoid of functional plasma membrane ("chemical skinning") has opened up new approaches for investigation of the contractile apparatus and its regulation (e.g., Filo et al., 1965; Endo et al., 1977; Gordon, 1978; Saida and Nonomura, 1978; Cassidy et al., 1981; Peterson, 1980, 1982a, 1982b; Sparrow et al., 1981). These studies have shown that skinned smooth muscle preparations produce graded isometric force in the range of Ca++ concentrations of about 10$^{-7}$ to 10$^{-5}$ M, and that the activation is accompanied by increased phosphorylation of the mol wt 20,000 myosin light chains. The Ca++ sensitivity is markedly increased by the addition of exogenous calmodulin, which is involved in the activation by Ca++ of the myosin light chain kinase activity (e.g., Adelstein and Eisenberg, 1980).

The question arises whether Ca++ can influence aspects of the crossbridge interaction other than the development of isometric force. A study of the dynamic mechanical properties of chemically skinned guinea pig taenia coli and rat portal vein (Arner 1982a, 1982b, 1983) showed that the force-velocity relation of the skinned preparation agrees with that of the intact muscle and revealed a marked Ca++ dependence of the maximal shortening velocity. This suggests that Ca++ regulates not only the number of activated crossbridges, but, possibly, also, kinetic properties of their interaction. Since each crossbridge cycle is supposed to be associated with breakdown of a specific amount of ATP, both of these aspects are reflected by the ATPase activity. A general relationship between actomyosin ATP turn-
over, shortening speed, and energetic tension cost in smooth muscle has been proposed by Ruegg (1971). It is, however, possible that quite different steps in the crossbridge interaction cycle are rate-limiting for isometric and isotonic contractile activity. The present study attempts to characterize further the factors influencing chemomechanical transduction in vascular smooth muscle. We have determined ATP turnover of intact and chemically skinned rat portal veins under conditions of graded contractile activation by Ca++. In addition, the length-tension relation was studied, as well as the ATPase activity in the skinned preparation, when active force is changed by changing muscle length. Some of the results have been reported in preliminary form (Hellstrand and Arner, 1982).

Methods

Preparation
Portal veins were dissected from male Sprague-Dawley rats weighing 200–300 g, cut open, and mounted longitudinally for isometric force registration under preloads of about 5.0 mN representing approximate optimal length (Lo). Force was measured by Grass FT03 force transducers, and registrations were made on a Grass polygraph. All experiments were done at room temperature (21–23°C). The lengths (5–7 mm) of all muscles were measured at the end of the experiment by using a dissecting microscope equipped with an ocular micrometer. Wet weight (1–3 mg) was determined by weighing on a Cahn electrobalance at the end of the experiment by using a dissection microscope after brief blotting on filter paper. Metabolic rates are reported normalized to tissue wet weight. Contractile force was related to tissue cross-sectional area, calculated assuming a density of 1.05 mg/mm³.

O₂ Consumption of Intact Portal Veins
For measurement of O₂ consumption, the vessels were mounted in an apparatus consisting of a glass chamber with an attached polarographic O₂ electrode, as described previously (Hellstrand and Paul, 1983), except that a stainless steel stopper with an O-ring seal against the chamber was used instead of a glass stopper. The volume of the chamber used in the present experiments was 0.25 ml. Force registration was by means of a 6 X 0 silk thread leaving the chamber via a stainless steel tube (length 32 mm, i.d. 0.6 mm) through the stopper on which the muscle was mounted. The chamber contents were stirred by a magnetic stirring bar, and the whole arrangement, including tubing and solution reservoir, was submersed in circulating water for temperature control (22°C).

The portal veins were mounted in a Tris-buffered solution of the following composition in mM: NaCl, 120; KCl, 6.0; MgCl₂, 1.2; CaCl₂, 2.5; glucose, 11.5; Na₂C₂H₃O₇, 0.026; and Tris (hydroxymethyl)aminomethane (Trizma Base, Sigma Chemical Co.) 23, titrated to pH 7.4. To reduce bacterial contamination, penicillin G (100 mg/ml) and streptomycin (300 mg/liter) were added to the solution. The solution was equilibrated with air. Muscles were preincubated overnight under 5 mN preload in a 50-ml organ bath before they were mounted in the chamber for O₂ measurements. The following sequence of measurements were made.

1. Nominally Ca++-free medium (composition as above, except that no CaCl₂ was added) 15 minutes.

2. High K⁺, Ca++-free medium (100 mM NaCl exchanged for KCl) 15 minutes. The muscles remained relaxed in this medium.

3. Addition of Ca++ by injection of 2–6 μl concentrated CaCl₂ into the chamber. Measurements continued for 30 minutes before the protocol was repeated from step 1 for another Ca++ concentration (2.5, 0.1, 0.2, 0.3, 0.4, 2.5, 10 mM).

With this protocol, registrations were obtained of basal O₂ consumption in the relaxed muscle (step 1), as well as continuous record of O₂ consumption and isometric force in the depolarized vein during relaxation (step 2) and contraction (step 3). The injection of a small amount of CaCl₂ into the chamber caused minimal disturbance of the O₂ record and allowed a high time resolution in measurement of O₂ consumption during the initial stages of the contracture. The P₀ in the chamber never decreased below 120 mm Hg. The first conditioning contracture in 2.5 mM Ca++ was not used in the subsequent analysis.

Chemically Skinned Preparations

The portal veins used in the chemical skinning experiments were mounted in normal medium, as above, except that no antibiotics were added. After a 2-hour accommodation, they were chemically skinned by overnight treatment with a solution containing 1% Triton X-100 (Gordon, 1978; Arner, 1982b). After the detergent treatment, the muscles were rinsed for 2 hours in a detergent-free medium before being transferred to a "relaxing solution" of the following composition: N-tris(hydroxymethyl)methyl-2-aminomethane sulfonic acid (TES) 30 mM; diethylolethylthiol, 0.5 mM; phosphoenolpyruvate, 5 mM; calmodulin, 1 μM (gift from Dr. Eva Thulin, Division of Physical Chemistry 2, Chemical Centre, University of Lund, Lund, Sweden); and pyruvate kinase, 20 U/ml. Free Mg²⁺ and MgATP, 2 and 3.2 mM, respectively, except as noted, were adjusted by adding Na₂ATP and MgCl₂. Ionic strength was adjusted to 0.15 with KCl and pH to 6.9 with KOH. A free-Ca++ concentration of 10⁻⁴ M was obtained by adding appropriate amounts of K₂CaEGTA and KEGTA while keeping the total EGTA concentration at 4 mM. "Activating solutions" of varying Ca++ concentrations were prepared by mixing the relaxing solution with one of similar composition but with a Ca++ concentration of 10⁻⁴ M. Free ion concentrations and ionic strength of the media were calculated by means of the method described by Fabiato and Fabiato (1979) with the equilibrium constants given by Fabiato (1981). Ca++ concentrations are reported here as their negative logarithms (pCa). In experiments in which sodium azide and ouabain were used, pH was checked after addition of the drugs.

The skinned preparations were mounted in plastic cups fixed on vertical stands rotating at 0.5 Hz for stirring. The incubation volume was 0.3 ml. Usually four muscles were mounted simultaneously. The cups were exchanged at 5- or 10-minute intervals, and, at the end of experiments they were frozen, together with solution blanks, and stored at −80°C until analysis.

Analysis of ATP Breakdown

The analysis of ATP breakdown was performed essentially as described by Peterson (1980). In principle, the ADP released by the muscle into the incubation medium is rephosphorylated to ATP by the action of the phosphoenolpyruvate and pyruvate kinase present. As a result, pyruvate is liberated in a stoichiometric relation to the
rephosphorylated ADP. The assay for pyruvate was performed by NADH-linked enzymatic analysis in a Farrand A-4 fluorimeter, as described by Lowry and Passonneau (1972). After the final fluorimeter reading in the pyruvate assay, myokinase (5 U/ml) was added and a further reading made to determine AMP (2 ADP produced in the myokinase reaction per AMP present). Usually, AMP release by the muscle accounted for 0 to 5% of the total observed ATP breakdown.

Statistics

Statistical evaluations were made by Student's t-test for paired and unpaired observations. Values are reported as means ± SEM, with the number of observations within parentheses.

Results

The intact portal veins used for measurement of O₂ consumption (J₀₂) were preequilibrated in the buffer medium overnight. In preliminary experiments, where muscles were equilibrated for only 2 hours before measurements, active force was higher than in the present series of experiments, but the relation between force and J₀₂ was the same. However, the overnight preincubation was adopted due to the long duration of the O₂ measurements (7–8 hours). With this procedure, reproducible J₀₂ in relaxed muscles was obtained throughout the subsequent metabolic measurements. J₀₂ in Ca⁺⁺-free normal and high K⁺ medium was 0.091 ± 0.010 and 0.091 ± 0.010 μmol/min per g, respectively (n = 6). The time courses of force and suprabasal J₀₂ (ΔJ₀₂ = increase in J₀₂ above that in high-K⁺ Ca⁺⁺-free medium) in contractions elicited by addition of 0.4 and 2.5 mM Ca⁺⁺ are shown in Figure 1. At the lower Ca⁺⁺ concentration (upper panel), force gradually increases during the 30-minute contraction. In contrast, ΔJ₀₂ is transiently increased during the first 5–10 minutes, and then declines toward approximately 50% of the peak value. On return to Ca⁺⁺-free normal medium, force and J₀₂ return to baseline levels. ΔJ₀₂ could not be followed with certainty until about 5 minutes after the solution exchange. After this point in time, there was no change in J₀₂ in the relaxed state. The limitation on the time resolution of J₀₂ measurements does not apply to the initial period of contraction, since Ca⁺⁺ was added without solution exchange (see Methods). At the higher Ca⁺⁺ concentration (lower panel), force rises quickly to an early peak value and then declines somewhat before it is gradually restored. ΔJ₀₂ shows a transient increase which is more rapid and pronounced than at the lower Ca⁺⁺ level.

J₀₂ values can be converted into ATP turnover (J_ATP) by using a factor of 6.4 (Hellstrand and Paul, 1983). In relaxed muscles, J_ATP is thus obtained as 0.58 μmol/min per g. Values for suprabasal J_ATP (ΔJ_ATP) of contracted muscles are shown in Figure 2. The open squares show J_ATP measured at 5 minutes after initiation of contraction plotted against the active stress at this point in time. The open circles show data obtained in the stable phase at the end of the 30-minute period. For both sets of data, the points fall on straight lines with correlation coefficients of 0.99. The slope of the line relating J_ATP to force at 5 minutes is 1.8 times that of the line in the stable phase.

The time course of ATP utilization during a sustained contracture of a chemically skinned portal vein activated at pCa = 4.5 in the presence of 1 μM calmodulin is shown in Figure 3. The measured values of ATP breakdown (J_ATP) are placed in the middle of the measurement periods (crosses). It is seen that activation results in about a 2-fold increase in J_ATP, compared with the relaxed state, and that this increase is maintained for as long as contraction continues. When J_ATP was measured in six muscles during four subsequent 10-minute periods at pCa = 4.5, values of 0.28 ± 0.03, 0.31 ± 0.06, 0.31 ± 0.04, and 0.31 ± 0.04 μmol/g per min were obtained. There was thus no transient increase in J_ATP of the skinned muscle during the early stages of contraction. Using the “Ca-jump” method, the rate of tension development in skinned preparations can be increased (Peterson, 1982b). The present data do not exclude the possibility that a transient high J_ATP could be present under such conditions. It is possible that an increased J_ATP during the development of tension could result from the changing mechanical
min«g

A-

0.3-

0.2-

0 1-

0 0

AJ

ATP

10 15

mm

FIGURE 2. Suprabasal ATP turnover ($\Delta J_{ ATP}$) calculated from $\Delta J_{ ATP}$ vs. isometric stress ($P$) in intact portal veins (open symbols, $n = 6$). Squares show values measured at 5 minutes after initiation of contraction (regression line slope $\Delta J_{ ATP}/P = 0.0250$, $r = 0.994$), whereas open circles show values at plateau of contraction (25–30 minutes, $\Delta J_{ ATP}/P = 0.0132$, $r = 0.988$). Filled circle indicates mean values of stress and $\Delta J_{ ATP}$ in skinned portal veins at $pCa = 4.5$ ($n = 30$).

state alone when $Ca^{++}$ level and myosin phosphorylation are already optimal.

Although the preparation shown in Figure 3 returned to the same $J_{ ATP}$ on relaxation as that measured before contraction, there was sometimes a markedly lower $J_{ ATP}$ in the final relaxation period. A corresponding finding in hog carotid artery has been reported by Peterson (1980). Several factors may be responsible for the ATPase activity measured in the relaxed preparation. The possibility that the assay was influenced by pyruvate released by the muscle was excluded, since no pyruvate could be detected when the muscles had been incubated in the absence of phosphoenolpyruvate and pyruvate kinase. Furthermore, no ATPase activity was detected after omission of MgCl$_2$ (free-Mg and MgATP = 0) or Na$_2$ATP (MgATP = 0) in the incubation media. This shows that the substrate for the measured basal ATPase activity is MgATP provided by the ATP-regenerating system in the medium. Since ATPases other than those of the contractile proteins may be present, the mitochondrial inhibitor sodium azide (1 and 5 mM) and the Na$^+/K^+$ pump inhibitor ouabain (1 mM) were tested. Neither basal ($pCa = 9$) nor maximally activated ($pCa = 4.5$) $J_{ ATP}$ could be reduced with either treatment. No $O_2$ consumption could be detected in skinned muscles.

The mean values of isometric force and $J_{ ATP}$ at $pCa = 4.5$ obtained in all experiments of the present investigation are inserted into Figure 2 (filled circle, $n = 30$) to allow a comparison with the intact preparation. This point, representing the maximally activated skinned preparation, falls close to the regression line corresponding to measurements during the transient of high $J_{ ATP}$ in the intact muscle.

The force developed by the skinned muscles is lower than that of the intact muscles, as already discussed (Arner, 1982b, 1983). The force development and $Ca^{++}$ sensitivity in the presence of 1 $\mu$m calmodulin observed in the present study agreed with those obtained earlier. By electron microscopy of the skinned portal veins fixed in buffered glutaraldehyde, the preparations were seen to be uniform throughout, with disrupted cell membranes, nuclei, and mitochondria, indicating that there probably were no regions of the preparations that had escaped skinning (B. Uvelius, personal communication).

To characterize the conditions for study of the energetics of the skinned portal vein, $J_{ ATP}$ and force measurements were made during repeated exposures to $pCa = 4.5$ in four muscles. The results are as shown in Figure 4. As discussed above, basal $J_{ ATP}$ in $pCa = 9$ (filled squares) is high, initially, but is seen to settle to a constant value after the first

![Figure 4](https://example.com/fig4.png)
contraction. Force (filled circles) and \( J_{ATP} \) during contraction (filled triangles) both fall with each subsequent contraction. As shown in the inset, suprabasal \( J_{ATP} \) (\( \Delta J_{ATP} \)) decreases linearly with force. The results show that repeated contractions do not give reproducible force, and hence this investigation was designed to use one contraction in each skinned muscle. Based on the results of the experiment, we also chose consistently to evaluate \( \Delta J_{ATP} \) relative to the stable \( J_{ATP} \) value in the relaxed muscle after contraction.

Figure 5 demonstrates the relation between \( J_{ATP} \) and developed force in the skinned portal vein at different \( Ca^{++} \) levels, as indicated by the pCa values inserted in the figure. The experiments were performed by stepwise increase in \( Ca^{++} \) at 20-minute intervals. \( J_{ATP} \) of the relaxed muscle (pCa = 9) was obtained after the contraction. The relation between force and \( J_{ATP} \) is clearly nonlinear. At pCa = 6.25, force is 60%, whereas suprabasal \( J_{ATP} \) (\( \Delta J_{ATP} \)) is only 23% of the maximal value at pCa = 4.5. Thus, the energetic cost of tension maintenance is lower in the region of lower \( Ca^{++}/force \) levels.

Force and \( J_{ATP} \) may be altered, not only by changing levels of activation by \( Ca^{++} \), but, also, by changing muscle length at constant \( Ca^{++} \) level. In the left panel of Figure 6 are shown values of active stress in intact portal veins (open circles) mounted at different lengths relative to Lo and activated directly by high \( K^+ \) medium (2.5 mM \( Ca^{++} \)). Skinned muscles activated at pCa = 4.5. Intact preparations were mounted at different lengths relative to optimal length (Ls) and then skinned at the same length. \( L_s \) denotes shortened length where no active force was produced. In the right panel, stress values are shown normalized to stress at \( L_s \). * = \( P < 0.05 \), ** = \( P < 0.01 \), ns = not significantly different.

The fact that basal \( J_{ATP} \) was the same as at the other lengths, and the long incubation periods used, would, however, speak against this possibility. Values of \( \Delta J_{ATP} \) obtained in muscles activated at short lengths giving no isometric tension (\( L_s \)) are also

![Figure 5. ATP turnover (\( J_{ATP} \)) vs. active stress (P) of skinned preparations in 8-12] at different \( Ca^{++} \) concentrations. The \( Ca^{++} \) levels are shown as pCAs.
shown in the figure. They do not differ from the values obtained at finite muscle length.

The experiments described in Figure 6 were done on separate sets of muscles at each length, due to the limitations on reproducibility that were described, above, in connection with Figure 4. In order to more accurately detect a possible variation of $J_{\text{ATP}}$ with muscle length, the following experiment was undertaken. Portal veins were contracted at $pCa = 4.5$ for 10 minutes, and then shortened so that they developed no active force, while being kept in the activating solution for a further 10 minutes. The results are shown in Figure 7. Whereas the activated muscles had a significantly lower ($P < 0.001$) $J_{\text{ATP}}$ at slack length than at $L_0$, $J_{\text{ATP}}$ in the relaxed state at $pCa = 9$ was lower still ($P < 0.001$). This demonstrates that muscle length influences $J_{\text{ATP}}$, as well as active force, but that reduction of force development by shortening the muscle is not sufficient to reduce $J_{\text{ATP}}$ at maximal activation to that of the relaxed state.

**Discussion**

A linear dependence of energy turnover on isometric force has been a consistent finding in intact striated and smooth muscles (Paul, 1980). In the intact rat portal vein, the slope of the relation between $J_0$ and force is dependent on the mechanical characteristics of contraction (Hellstrand, 1977). In phasic spontaneous contractions of short duration, the relation was steeper than in tonic high $K^+$-induced contractures. In later investigations by Arner and Hellstrand (1980) and by Hellstrand and Paul (1983), the initial stages of a maintained high $K^+$ contracture were shown to be associated with a higher rate of ATP turnover than were the later stages. Similarly, a lower maximal shortening velocity ($V_{max}$) in maintained vs. phasic contractions was demonstrated by Uvelius and Hellstrand (1980). A possible mechanism to account for this variability in shortening velocity and tension-dependent metabolism is that the rate of crossbridge turnover can somehow be regulated in the smooth muscle, allowing a specific force maintenance to be associated with different rates of ATP turnover. Murphy and associates (Dillon et al., 1981) have suggested that the rate of crossbridge turnover is regulated by the state of phosphorylation of the mol wt 20,000 myosin light chains. The myosin light chain kinase which catalyzes the phosphorylation process is regulated by the $Ca^{++}$-calmodulin complex (e.g., Adelstein and Eisenberg, 1980). It is thus possible that $Ca^{++}$ can be involved in the regulation of crossbridge cycling rate, not simply as an on-off switch, but in a more complex way. An influence of $Ca^{++}$ on crossbridge cycling rate is suggested by the finding that the maximal shortening velocity of the skinned portal vein increases with increasing $Ca^{++}$ (Arner, 1983).

The purpose here was to investigate aspects of crossbridge turnover estimated from the ATP flux and isometric force. Transients in $J_0$, were demonstrated during slowly developing contractures elicited in depolarized portal veins at 22°C by addition of different amounts of extracellular $Ca^{++}$ (Fig. 1). These findings correspond to those previously reported (Hellstrand and Paul, 1983), although the time course is slower, due to the lower temperature. The metabolic tension cost estimated at peak and steady state $J_0$, differed by a factor of 1.8 (Fig. 2). This difference in tension cost corresponds well to the observed variation in $V_{max}$ during the course of contraction (Uvelius and Hellstrand, 1980; Dillon et al., 1981). The experiments on skinned muscles reported here could bear on the possibility that intracellular $Ca^{++}$ variation is a mechanism underlying the alterations in mechanical and metabolic rates. In chemically skinned portal veins, activated at constant $Ca^{++}$ force and $J_{\text{ATP}}$ both remained at constant levels throughout the contraction (Fig. 3). The force-velocity relation has also been shown to be the same at early and late stages of the force plateau in the skinned portal vein (Arner, 1983). Thus, when $Ca^{++}$ is held constant, which is feasible in the skinned preparation, no transients in mechanical or metabolic kinetics are detected.

Despite reduced force with repeated contractions, which is a common problem in studies on skinned smooth muscles, basal $J_{\text{ATP}}$ (after the first contraction) as well as the relation between $\Delta J_{\text{ATP}}$ and force (tension cost), remained invariant (Fig. 4). Basal $J_{\text{ATP}}$
was about one-third of that estimated from \( J_0 \) in the intact preparation. The cause of the basal ATPase activity in the skinned relaxed muscle is unknown. Since azide or ouabain did not reduce this ATP turnover, it is unlikely that mitochondrial or Na\(^+\)/K\(^+\) pump activity contributes. The elevated \( J_{ATP} \) of some muscles in relaxing solution initially after skinning suggests that some energy-dependent process, perhaps related to Ca\(^++\) handling, is operating but is successively inhibited. The recent demonstration of regenerative Ca\(^++\) release in preparations briefly skinned with saponin (Haeusler et al., 1981; Saida, 1982) suggests a mechanism for Ca\(^++\) sequestration, although the present overnight treatment with Triton X-100 would be expected to destroy intracellular Ca\(^++\) stores much more drastically.

A nonlinear relation between \( J_{ATP} \) and active stress appears when Ca\(^++\) is varied in the skinned preparation (Fig. 5). A similar dissociation between \( J_{ATP} \) and force at supramaximal Ca\(^++\) seems to be present in skinned taenia coli, as recently reported (Guth and Mrwa, 1982; Hellstrand and Arner, 1983). Several processes may contribute to the ATPase activation by increasing Ca\(^++\). If myosin-actin interaction is the main site of ATP turnover, the results suggest a nonlinear relation between total crossbridge turnover and developed force. At higher Ca\(^++\), each crossbridge cycle would then be less efficient in producing force, perhaps as a result of an increased cycling rate. In addition to crossbridge interaction, however, energy-dependent Ca\(^++\) translocation, as discussed above, may occur when Ca\(^++\) is added. It is notable that a significant accumulation of Ca\(^++\) into mitochondria of saponin-skinned rabbit portal-anterior mesenteric vein at pCa = 5 has been found by Somlyo et al. (1982) using electron probe analysis. At pCa = 6, which gave almost maximal force, no such Ca\(^++\) accumulation was seen. This would seem to be a less likely explanation for the Ca\(^++\)-induced ATPase in the present Triton-skinned preparations. Moreover, azide did not reduce \( J_{ATP} \) of muscles activated at pCa = 4.5. Phosphorylation of muscle proteins is another possible cause of ATP usage. Based on myosin content of the portal vein and the rate of dephosphorylation estimated in the model study on taenia coli by Peterson (1982a), the rate of ATP usage associated with phosphorylation-dephosphorylation of myosin at steady state is on the order of 0.003 \( \mu \)mol/min per g, and thus negligible in this context. An interesting conclusion to be drawn from the curvilinear relation between \( J_{ATP} \) and force with varied Ca\(^++\) is that the decreased force in repeated contractions is unlikely to be caused by Ca\(^++\) desensitization, since the \( \Delta J_{ATP}/P \) relation is linear when force decreases in successive contractions (Fig. 4). The force and \( \Delta J_{ATP} \) obtained in our total material of skinned portal veins maximally activated at pCa = 4.5 falls close to the regression line representing the relation between \( J_{ATP} \) and force in the living muscle early in the contraction during the transient of high energy turnover (Fig. 2). The contractile energetics of the skinned muscle when maximally activated by Ca\(^++\) thus corresponds to the peak rather than to the steady state metabolic turnover rate during a sustained contracture in the living muscle. Together with the curvilinear relation between force and \( J_{ATP} \) (Fig. 5), this could agree with the possibility that variations in intracellular Ca\(^++\) are responsible for the observed variability in metabolic and mechanical kinetics via an influence on crossbridge turnover rate.

The structural basis of the length-force relation in smooth muscle is as yet unclear, although its general similarity to that of skeletal muscle implies a sliding filament mechanism of contraction (Murphy, 1980). Figure 6 (right panel) demonstrates that the skinned portal vein at lengths below \( L_o \) is capable of developing higher relative force than the intact muscle at the same length. A similar discrepancy between the length-force relations of intact and skinned skeletal muscle has been described (Schoenberg and Podolsky, 1972). It is thus possible that, in smooth as in striated muscle, an inactivation of contraction at short muscle lengths is present due to a depression of excitation-contraction coupling (Taylor and Rüdel, 1970). The rate of ATP turnover of the skinned portal veins decreased at lengths below \( L_o \) (Fig. 6, left panel), although, relative to active force, the decrease is much less prominent than when Ca\(^++\) is varied (cf Fig. 5). At 1.3 \( L_o \), \( \Delta J_{ATP} \) was not significantly different from the value at \( L_o \), but—if anything—seemed to be larger, whereas force was reduced by 44% relative to \( L_o \). The technical problems associated with the stretched preparation are discussed in Results, although the possibility cannot be excluded that an acceleration of energy turnover is present in the stretched skinned muscle. This result contrasts, however, with intact vascular smooth muscle, where \( J_0 \) has been found to decrease at lengths above \( L_o \) (Paul and Peterson, 1975).

In skinned muscles shortened during a maximally activated contraction, there is a statistically significant reduction of \( J_{ATP} \) (Fig. 7). However, the component of ATP turnover associated in this way with force development is smaller than the change in \( J_{ATP} \) on activation from pCa = 9 to pCa = 4.5 at \( L_o \). This could suggest the presence of Ca\(^++\)-induced ATPase activity not related to force development as discussed above. However, myosin-actin interaction in the fully shortened muscle cannot be excluded a priori on the basis of no external force output, since internal resistive forces might be of importance. In intact and chemically skinned hog carotid artery, Pfizter et al. (1982) report a linear decrease in stiffness with force as the muscle was shortened. In the skinned preparations, the stiffness-force relation, when extrapolated to zero force, indicated some residual stiffness, which could suggest internal force generation in the shortened activated muscle or,
alternatively, the presence of attached non-force-generating crossbridges. Non-actin-activated myosin ATPase activity could possibly contribute to \( J_{ATP} \) of the activated shortened muscle if myosin-actin interaction is indeed eliminated in this state. Influence of Ca\(^{2+}\) on ATPase activity of smooth muscle in the presence of actin has been observed in biochemical studies (Gorecka et al., 1976; Suzuki et al., 1978).

This study has demonstrated that the variation in crossbridge turnover rate inferred from the mechanical and metabolic kinetics of intact vascular smooth muscle can be reproduced in skinned preparations at controlled Ca\(^{2+}\) levels. The length-force relation of the intact smooth muscle may contain a component of deactivation at lengths below \( L_0 \).

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