72. Time-Resolved ^{31}P -NMR Studies on Contraction and Recovery of Bullfrog *Sartorius* Muscles

Mechanism of Post-Contractile Increase in NMR-Visible Phosphates

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The nature of the post-contractile phosphocreatine (PCr) utilization has been described in the preceding papers.^{1),2)} In the present paper the possible explanation of this phenomenon is discussed.

It has *a priori* been assumed that the energy produced by muscle contraction should match that released by the splitting of ATP, or PCr. Recent energy balance studies have shown, however, that a significant fraction of the observed heat plus work cannot simply be explained by PCr splitting, indicating that some reactions other than PCr splitting must be contributing to the energy output.³⁾⁻⁵⁾ Since there is no phosphagen utilized other than PCr by muscle,⁶⁾ this unexplained energy should be met by PCr utilization during the recovery period after contraction, or post-contractile PCr utilization.

It has been found difficult to prove post-contractile utilization of PCr by chemical analysis and the results are so far contradictory. Maréchal and Mommaerts⁷⁾ have provided evidence that PCr of about $1\ \mu\text{mol g}^{-1}$ is in fact utilized after contraction. Kushmerick and Paul⁸⁾ observed no PCr splitting up to 60 s after a 2 s tetanus nor up to 200 s after a 10 s tetanus. Kushmerick and Paul⁸⁾ employed, however, the mean of $\Delta P_i\ \text{g}^{-1}$, and $\Delta \text{PCr g}^{-1}$ as representing the quantity, $\Delta P\ \text{g}^{-1}$, for their experiments. Therefore it is difficult to disprove the post-contractile PCr splitting, because ΔPCr and ΔP_i follow different time courses as were shown in the present study. It was shown by Lundsgaard⁹⁾ that at 2°C about 30% of the PCr splitting which resulted from a 25 s isometric tetanus occurred during the 3 min after the contraction had ended. The amount of post-contractile PCr splitting is larger than that in our study.²⁾ Spronck¹⁰⁾ also reported a break-down of PCr of an order of $0.5\ \mu\text{mol per g}$ muscle during the minute following five twitches at 2°C . Although Spronck¹⁰⁾ has discussed seasonal effects, our results²⁾ showed no evidence of such effects. Kushmerick and Paul¹¹⁾ have reported that for a series of isometric tetanic contractions having nearly identical tension-time integrals, recovery oxygen consumption was dependent on the interval between tetanii and reached a maximal value for intervals equal to or greater than 200 s. Together with the results on initial chemical reactions and with the initial maintenance heat production, the results in the preceding paper²⁾ indicated a saturable non-steady state reaction, which is reversed in the post-contractile period utilizing less than about $1\ \mu\text{mol ATP per g muscle}$.^{8),11)-13)}

Dawson, Gadian and Wilkie⁶⁾ using ^{31}P NMR showed that approximately

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5 $\mu\text{mol g}^{-1}$ of PCr was broken down as a result of a 25 s tetanic contraction of frog sartorius muscles at 4°C and this is approximately equaled by the increase in inorganic orthophosphate (P_i). Since it was necessary to accumulate spectra for 7 min, it must have been difficult to observe the course of changes, whose time constant is 4 to 5 min (see Figs. 1b and 1d in ref. 2)). Using a time-resolved ^{31}P NMR we showed that after complete relaxation of bullfrog sartorius muscles²⁾ and of toad sartorius muscles as well,¹⁴⁾ sufficient PCr was not utilized to account for P_i increase. Subsequently the level of PCr decreased in the early post-contraction period, whereas the decrease of PCr was not accompanied by a concomitant appearance in P_i . It should be stressed that, using the time-resolved ^{31}P NMR, the temporal separation between P_i released and PCr utilized was first realized.

The next problem would be to identify the mechanism that causes this separation, or the post-contraction splitting of PCr. Because the amount of P_i released without PCr utilization almost coincides with the amount of myosin heads in muscle (0.28 mol g^{-1}),¹⁵⁾ it is quite possible that the appearance of P_i without PCr utilization, or the delayed splitting of ATP, is closely associated with the acto-myosin ATPase mechanism. The temporal separation between the extent of P_i released and that of PCr utilized, or the delayed splitting of PCr in the post-contraction period, could be explained if a large proportion of myosin heads (S1) is in the form of acto-S1-ADP complex in a steady state of contraction.¹⁴⁾ Thus, the present results may indicate that on deactivation the rate of release of ADP from myosin is very slow at low temperatures. Results obtained by biochemical studies on myosin ATPase mechanism indicates this to be at least possible, because the rate of release of ADP from myosin S1 shows a marked temperature dependence.¹⁶⁾ At 10°C the rate of delayed splitting of PCr is accelerated as indicated by the results described above.

If the phenomenon of the post-contraction utilization of PCr is really associated with the acto-myosin ATPase cycle, it would decrease linearly with increase in length of the muscle. It was not possible to prove this on sartorius muscles because active tension produced could be maximally reduced by only one third due to the relative unextensibility of these muscles. However, in our preliminary results on semitendinosus muscles, which can be stretched till there is almost no overlap between the filaments, the post-contraction utilization of PCr was greatly reduced on stretching the muscles maximally.¹⁷⁾

The activity of creatine kinase during contraction at 4°C is large enough to account for the well-established observation that, during contraction, the concentration of ATP does not change by more than 2–3%.¹⁸⁾ The content of creatine kinase in frog skeletal muscle is at least 0.175 $\mu\text{mol g}^{-1}$, which is comparable to that of myosin (see above).^{15), 19)} However, forward and backward fluxes in creatine kinase reaction are not much altered in active muscles compared with those in resting muscles.¹⁸⁾ Therefore, there is no way in which a lag in creatine kinase reaction can contribute appreciably to the post-contraction utilization of PCr.

It has been shown that during a short isometric tetanus in frog muscle a large fraction (60%) of Ca^{2+} in the terminal cisternae of the sarcoplasmic reticulum is released and Ca^{2+} in the myoplasm is raised to the concentration sufficient to saturate the Ca^{2+} -binding sites on troponin and on parvalbumin.²⁰⁾ After relaxation parvalbumin is saturated with Ca^{2+} , the half-time for the return of Ca^{2+} to the terminal cisternae during the early recovery period after a tetanus

is approximately 1 s at room temperature.²⁰⁾ Therefore, it is likely that the course of recovery of both PCr and P_i should to some extent be influenced by the Ca^{2+} -pump operating early in the recovery period. However, the post-contractile PCr splitting is not accompanied by a concomitant increase in P_i . It is very unlikely that during the maintained activity of Ca^{2+} -ATPase very little P_i is released while ATP is continuously utilized.

Stimulation of glucose utilization may be expected through the activation of phosphorylase b kinase.^{21),22)} This mechanism is, however, not very likely an explanation because peaks attributable to sugar phosphates did not show any appreciable increase in the post-contractile period after short contractions. The other possible mechanism would be phosphorylation of myosin light chain caused by the contractile activity.^{23),24)} According to Bárány and Bárány,²³⁾ maximally 0.35–0.45 mole per mol of 18,000-dalton myosin light chain can be phosphorylated during a full activation of frog skeletal muscles at various temperatures. These amounts of phosphates incorporated would correspond to 0.1–0.13 μmol phosphate per g muscle, which is about one third of the amount of the post-contractile PCr utilization. Moreover, phosphorylation of the light chain takes place during the activation of contraction and not during the post-contractile period.²³⁾

As described above, at the actomyosin, PCr accepts ADP to generate ATP. On the other hand, at the mitochondria, ATP accepts creatine to produce PCr. It is thus possible as an alternative explanation that at mitochondria NMR-invisible phosphorus of ATP, which is bound to the mitochondrial creatine kinase, becomes NMR-visible phosphorus of PCr.

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