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IN VIVO ^{31}P NMR IN CRUSTACEAN MUSCLES : FATIGUE AND RECOVERY IN THE TAIL MUSCULATURE FROM THE PRAWN PALAEMON ELEGANS

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SUMMARY: ^{31}P NMR has been used to observe the in vivo phosphometabolite concentrations in the tail musculature from the prawn Palaemon elegans, at rest and after escape swimming and subsequent recovery. Muscular fatigue corresponds to a 60 % breakdown of phosphoarginine, and a 45 % increase of sugar phosphates. The pHi fell from 7.10 to 6.86. During recovery, the sugar phosphates and arginine phosphate are replenished after 20 minutes. The ATP concentration did not change throughout the experiment. The pHi was restored within 20 minutes.

The mechanisms responsible for the fatigue development in muscle remain essentially unknown. Probably, there is no single cause, and fatigue may take place both at central and peripheral sites independently. Peripheral sites, located within the muscle cells, include various steps of muscle excitation-contraction coupling which are not clearly defined. ^{31}P NMR has been used to relate the mechanical manifestation of fatigue to the simultaneous biochemical and energetic changes in vertebrate muscles (1). Different types of Molluscan muscles have been examined using ^{31}P NMR (2-5), but only a small number of studies on Crustacean muscles have been investigated up to now (6-9). In addition, the contraction and recovery of crustacean tail muscle and the fatigue development during whole body work were never studied by this method.

The Crustacean abdominal muscles are employed either for maintaining the posture of the animal, or for performing very quick movements, propelling the animal backward. Thereafter, the muscles remain inactive for a few minutes. Thus, the tail beats need a large energy production within a very short time.

We report here the patterns of phosphometabolite changes in the Palaemon elegans tail musculature after an exhaustive work, and the kinetics of the subsequent restoration to the resting state, studied by ^{31}P NMR on intact animals.

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MATERIALS AND METHODS

Animals: Specimens of Palaemons elegans were collected in the bay of Concarneau, Brittany. Whole animals were placed in 10 mm (i.d.) NMR tubes in 15 ‰ filtered seawater. A peristaltic pump was used to pump solution into the NMR tube, at a rate of 3 ml/min.

NMR spectra: NMR tubes were maintained at 11-13°C by passing cooled air around the sample tube. ^{31}P NMR spectra were taken using a pulsed Fourier transform mode at 40.32 MHz on a Jeol FX100Q spectrometer. Typically, 200 data acquisitions were accumulated with a delay time of 3.0 sec, a tip angle of 72° (pulse width 60 μ sec) and a sweep width of 2500 Hz. Chemical shifts reported in ppm were measured relative to an external standard of 85 % phosphoric acid. The internal standard used was the chemical shift of arginine phosphate which is pH independent in the physiological range (pH 6-8).
Saturation factors, described by Dawson et al (1), necessary to compare the relative levels of the different compounds, were obtained for Palaemon elegans tail muscles by measuring two spectra from 100 acquisition data sets using two different delay times (3 and 12 sec). Since the tip angle was of 72°, the saturation factor was 1.0 for arginine phosphate and ATP. We used a freshly dead prawn to determine the saturation factor for Pi (1.31), due to the small size of the peak in living control animals.
The chemical shift of Pi versus pH was observed by titrating one solution of 38.3 mM taurine, 1.4 mM aspartate, 3.9 mM alanine, 3.7 mM glutamate, 10 mM K_2HPO_4, 5 mM EDTA, 3 mM arginine phosphate and isoosmotic KCl with respect to hemolymph (536 mosm/ kg H_2O), reflecting the amino acid composition of the caudal muscles, as determined by Richard (10).

RESULTS

Fig. 1a shows a ^{31}P NMR spectrum obtained in vivo from the abdominal section of a prawn. The major resonances arise from "sugar phosphates" (SP) at 7.31 ± 0.70 ppm, inorganic phosphate (Pi) at 5.37 ± 0.31 ppm, arginine phosphate (Arg-P) at 0 ppm, integrated intensities of ATP and ADP, at -2.03 ±

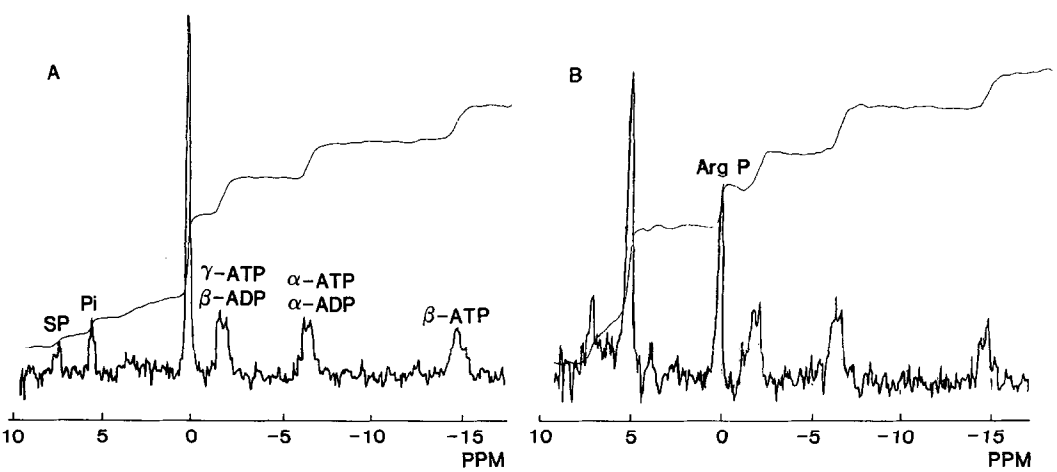

Fig. 1. ^{31}P NMR spectra of Palaemon elegans tail muscles in controls (A) and exhausted animals (B). Each spectrum is average of 200 scans taken at 3.0 sec. intervals.
S.P.: Sugar phosphates, Pi: inorganic phosphate, Arg-P: arginine phosphate, γ, α and β resonances of ATP.

TABLE 1
RELATIVE PEAKS OF RESONANCES

	SP	Pi	Arg-P	γ-ATP	α-ATP	β-ATP
Control	0.044 ± 0.010	0.144 ± 0.017	0.459 ± 0.072	0.135 ± 0.027	0.134 ± 0.027	0.113 ± 0.026
Exhausted	0.097* ± 0.025	0.385** ± 0.063	0.179** ± 0.026	0.124 ± 0.018	0.125 ± 0.080	0.090 ± 0.031

Values are means ± S.E. for integrals taken from spectra of 200 scans, normalyzed to the sum of all peak integrals. Significant differences ($\alpha < 0.05$: *, $\alpha < 0.01$: **) are calculated by the non parametric test of Mann Whitney.

0.13 ppm, and at -7.17 ± 0.06 ppm, and β phosphorus atoms of ATP, at -15.85 ± 0.28 ppm. In order to identify the peaks observed in the sugar phosphates region of the spectra, ^{31}P NMR spectra of AMP and IMP were taken *in vitro* at pH 7.4. IMP and AMP had a chemical shift of 6.68 and 7.09 respectively. The level of Pi and of SP was very low, and in many experiments we could not detect SP at all. On the other hand, the tail muscles contained a large quantity of arginine phosphate.

The powerful contractions of the tail muscles effecting the tail beats caused rapid fatigue as indicated by the decreasing power of muscle contractions. After performing about 40 tail flips over 1 min, the prawns did not respond to the stimuli. Large changes in metabolite concentrations occured

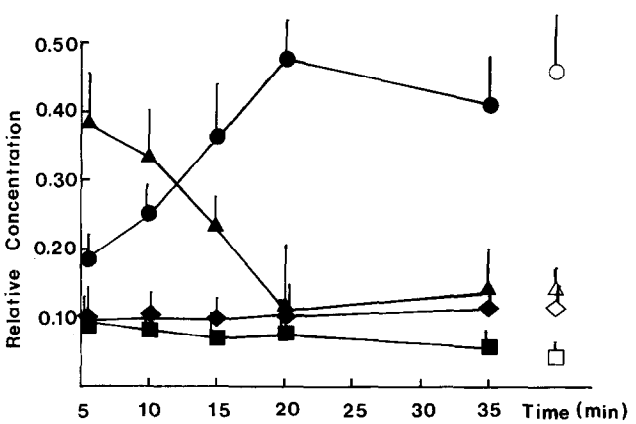

Fig. 2. Recovery of *Palaemon elegans* tail muscles from exhaustive work. Values are means ± S.E. for integrals taken from spectra of 200 scans, normalized to the sum of all peak integrals. (●), arginine phosphate; (▲), inorganic phosphate; (◆), ATP; (■), sugar phosphates.
Open symbols represent average of control values obtained from resting animals before stimulation.

TABLE 2
EVOLUTION OF THE SUM ARG-P + Pi

	Control	Recovery				
		5min	10 min	15 min	20 min	35 min
Arg-P + Pi	0.602 ± 0.024	0.564 ± 0.023	0.584 ± 0.015	0.590 ± 0.029	0.589 ± 0.026	0.571 ± 0.021

Values are means ± S.E. for integrals taken from spectra of 200 scans, normalized to the sum of all peak integrals.
No significant differences were shown by the non parametric test of Mann and Whitney.

after exhaustive work. This is demonstrated in Fig. 1b which shows a spectrum of an abdominal section after exhaustion. Arginine phosphate fell to about 60 % its resting level and there were large increases in Pi and sugar phosphates while ATP remained constant (table 1).

Changes taking place during recovery from exhaustive work are shown in Fig. 2: the arginine phosphate and Pi concentrations normalize within 20 min. Sugar phosphates increased progressively within 30 min. ATP remained constant. The increase in Pi, immediately after exhaustive work, was roughly equivalent to the phosphate released upon hydrolysis of arginine phosphate, and the restoration of arginine phosphate was balanced by the decrease in Pi, during the recovery period (Table 2).

In order to characterize the extent of energetic changes caused by exercise, various indices have been calculated (table 3) : the average ratio of Arg-P to the β peak of ATP, the Arg-P/Pi ratio, and the NMR index: (ATP +

TABLE 3
RATIOS OF KEY PHOSPHATE METABOLITES

	Control	exhausted	Recovery			
			10 min	15 min	20 min	35 min
$\frac{\text{Arg-P}}{\text{ATP}}$	4.49 ± 2.29	2.25** ± 1.02	2.56** ± 0.29	3.79 ± 1.08	5.39 ± 1.68	4.13 ± 1.03
$\frac{\text{Arg-P}}{\text{Pi}}$	3.15 ± 0.70	0.48** ± 0.12	0.78** ± 0.21	1.56** ± 0.38	3.74 ± 1.59	2.91 ± 1.21
$\frac{\text{Arg + ATP}}{\text{Arg-P + Pi + ATP}}$	0.80 ± 0.03	0.41** ± 0.07	0.51** ± 0.07	0.66** ± 0.04	0.84 ± 0.10	0.77 ± 0.08

Each value is a mean ± S.E.. Significant differences ($\alpha < 0.01$ **) were calculated by the non parametric test of Mann and Whitney.

Arg-P) / (ATP + Arg-P + Pi), proposed by Lavanchy et al. (11). The three indices dropped with exhaustive work and returned to the initial level within 20 min of recovery.

The intracellular pH (pHi) for resting Palaemon elegans tail muscles was 7.10 ± 0.06, at 11-13°C. Exercise produced significant changes in the pHi of the tail musculature : the pHi fell to 6.86 ± 0.07, within 5 min, for a total reduction of 0.24 unit. The restoration took place gradually within 20 min.

DISCUSSION

^{31}P NMR has the advantage of providing many simultaneous determinations of phosphometabolites for each exercise bout and during the recovery period without altering the integrity of the animal. The peak labelled sugar phosphates, at about 7.3 ppm, contains resonances from hexose and triose phosphates. IMP and AMP have a chemical shift in the same region from in vitro titration data. However, the "sugar phosphates" are probably essentially represented by fructose-1,6-bisphosphate which is known to increase during the muscular activity in prawns (12) as a consequence of increased glycogen utilization.

The tail musculature of the prawn Palaemon elegans contains a large quantity of phosphoarginine with correspondingly low levels of Pi (as shown by the high ratio Arg-P/Pi in resting animals). During exhaustive work, the muscle phosphagen content was diminished by about 60 % and was balanced by the changes in Pi. Rapid tail flipping leads at first to utilisation of ATP obtained from arginine phosphate hydrolysis. The ATP consumption rate is found to be very high in crustaceans exercised maximally for a short period (12). The initial decline in muscular force in man is, at least in time, related to the depletion of the phosphocreatine stores. Inorganic phosphate is present in muscle cells at millimolar concentration and increases several fold during activity. Accumulation of inorganic phosphate could explain the fatigue development in terms of cross-bridge kinetics (13). However, both ATP decrease and H^+ accumulation could be responsible for the large decrease in force production (14).

In Palaemon elegans, the decline in force was observed after about 30 tail flips. After, they responded more and more weakly to the stimuli until they were fully exhausted. It is clear that depletion of arginine phosphate is not the cause of fatigue in the abdominal muscle of P. elegans. During the second less powerfull series of tail beats, anaerobic glycolysis is essentially used as ATP source. At the same time, energy charge has fallen.

Onnen and Zebe (12) demonstrated in Crangon crangon a drop in ATP concentration after exhaustive work and a return to its normal value within 5 min. Such rapid changes could have been overlooked in our NMR measurements,

the acquisition time being 10 min. ATP decrease occuring when force is declining could be the cause of fatigue in P. elegans. In vertebrates, during ATP degradation, only minor increases of ADP content were observed and the ADP was apparently broken down via AMP to IMP by the AMP deaminase reaction. In crustacean abdominal muscle, the very low activity of the AMP deaminase (unpublished results) should lead to the accumulation of ADP and AMP, reaching inhibiting levels and thus, developing fatigue. On the other hand, accumulation of Pi is known to inhibit the AMP deaminase reaction (15).

Phosphagen reserves are fully replenished after 20 min of recovery. This agrees with earlier observations with frog sartorius muscle NMR experiments (1). Shorter periods of recovery (1 min) are found in rat leg muscles NMR experiments (16). On the contrary, regeneration of phosphoarginine requires longer periods in the prawn Crangon crangon tail muscle (30 min), (12). The rapid decrease of sugar phosphates and restoration of arginine phosphate after exhaustive work in the prawn Palaemon elegans shows its high capacity of recovery from functional anaerobiosis.

The intracellular pH of Palaemon elegans resting tail muscles was 7.10 $\pm$ 0.06. The decrease in intracellular pH has long been considered to be one of the main causes of the development of muscular fatigue. Artificially induced acidosis in isolated rat muscle resulted in a decrease in isometric contraction force and prolongation of relaxation time as when the decrease in muscle pH was achieved by muscular activity (17). Intracellular acidification could decrease the contractile properties by inhibiting actomyosin ATPase activity, Ca^{2+} release and Ca^{2+} affinity of troponin (14) or by inhibiting glycolysis. However, glycolysis was found to proceed at pHi values as low as 6.81 in a molluscan adductor muscle (18).

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