Changes in intracellular pH and inorganic phosphate concentration during and after muscle contraction as studied by time-resolved ^{31}P-NMR

Alkalinization by contraction

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The changes in intracellular pH and concentration of inorganic phosphate (P_i) were studied during and after contraction of bullfrog sartorius muscles by using ^{31}P-NMR. The intracellular pH was 7.04 in resting muscles at 4°C and an alkalinization by as much as 0.08 pH unit occurred following 5 s tetanic contraction. During the recovery period after contraction the increased level of intracellular pH returned to the resting level, the time course of which coincided with that of the recovery of the P_i concentration. Based on the changes in the intracellular pH and P_i concentration, the buffering power was estimated to be 18 mM/pH unit.

^{31}P-NMR *Intracellular pH* *Alkalinization* *Frog muscle* *Recovery metabolism* *Buffering power*

1. INTRODUCTION

^{31}P-NMR is a powerful tool for studying the intracellular pH of muscles because the chemical shift of inorganic phosphate (P_i) relative to that of creatine phosphate (PCr) is a function of pH [1–4]. Since NMR spectroscopy is inherently non-destructive, both the time course of a changing intracellular pH and that of intracellular concentrations of phosphate-containing metabolites can be followed in the same muscles. Authors in [5] developed the physiological techniques of maintaining frog skeletal muscles alive for a long period and observed the changes of intracellular concentrations of metabolites following contraction with a time resolution of 7 min. An improved method for time resolution has led us to a more detailed study of time course. In fact, we observed recently the time course of the levels of P_i and PCr with a resolution of 16 s under well-oxygenated conditions [6].

Here, by using ^{31}P-NMR we determined the time course of the changes in intracellular pH in bullfrog skeletal muscles during and after isometric tetanic contractions under well oxygenated conditions. The results indicated an alkalinization of intracellular pH during contraction. The buffering power of cytoplasm was also estimated.

2. MATERIALS AND METHODS

Sartorius muscles of bullfrogs, *Rana catesbeiana*, were used. Sixteen muscles (wet wt ~5–10 g) were mounted at their lengths in situ on a muscle holder built in a 25-mm outer diameter NMR sample tube. Muscles were supplied with oxygen by superfusing at a rate of 17 ml/min at 4°C with oxygenated Ringer solution [composition in mM: NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) adjusted to pH 7.0 with NaOH, 6] bubbled with 95% O_2–5%

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CO_2. Muscles were stimulated in an NMR spectrometer magnet while the tension produced was monitored.

^{31}P NMR spectra were recorded at 81 MHz on a Bruker WM200wb widebore spectrometer with 5 kHz spectral width, 60° pulse width and 2 s recycling time. The probe temperature was kept at 4°C throughout the measurements. Eight transients were accumulated as a unit of observation and thus the time resolution of the measurements was 16 s. Muscles were stimulated 8–16 times at intervals of 30–60 min and the FIDs during the same preset time intervals phased with the stimuli were further averaged. The time courses of intracellular [P_i] and pH were fitted with single exponentials by the least-squares method.

3. RESULTS

Fig.1 shows the ^{31}P-NMR spectrum for bullfrog sartorius muscles in the resting state at the end of the recovery period after 5 s isometric tetanic contractions. Small peaks of sugar phosphates were detected but phosphodiesters were not found with this signal-to-noise ratio. The concentration of P_i was low as compared with that of PCr [6]. By a 5-s contraction, the peak of P_i was shifted to the lower field by about 0.1 ppm and was enlarged in its area (fig.2). Both the chemical shift and the area of the P_i peak returned to the resting level as recovery proceeded. The areas of P_i, corrected for 'saturation' caused by the intervals of radiofrequency pulses [6], were converted to concentrations by assuming that the resting PCr content was 27 mmol/kg [5]. The intracellular pH values (pH) were calculated from the chemical shifts of P_i (δ^{obs}) according to the following equation:

$$pH = pK_a + \log (\delta^{obs} - \delta_A)/(\delta_B - \delta^{obs})$$

where δ_A and δ_B are the chemical shifts of $H_2PO_4^-$

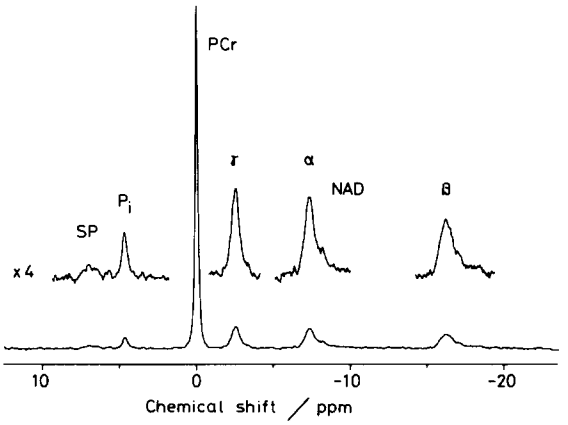

Fig.1. ^{31}P-NMR spectrum of bullfrog sartorius muscles, at the end of the recovery period after 5-s isometric tetanic contractions. SP, sugar phosphates; α, β and γ, ATP α-, β- and γ-phosphate. The peaks, except for PCr, are also shown in the scale enlarged 4-times vertically. Chemical shifts are expressed in ppm downfield from PCr. The spectrum is the result of 352-data accumulation [32 scans (4 units) times 11 contractions].

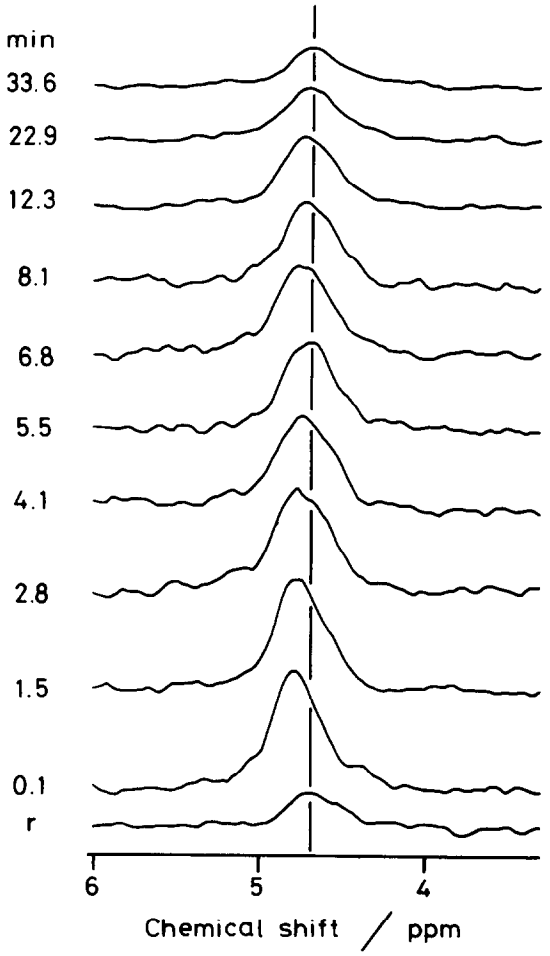

Fig.2. P_i region of ^{31}P-NMR spectra of the bullfrog sartorius muscles at the indicated minutes after 5-s isometric tetanic contraction. The recording indicated by 'r' is at the end of recovery; i.e., just before contraction. Vertical bars are drawn at 4.68 ppm.

and HPO_4^{2-}, respectively. The values of 6.88 (pK_a), 3.55 ppm (δ_A) and 5.6 ppm (δ_B) used for the calculation were the values determined under physiological conditions [5].

The intracellular pH of the resting muscles was 7.04 and increased by as much as 0.08 by the 5-s isometric tetanic contraction. Fig.3 shows the time courses of the intracellular [P_i] and pH after muscles have relaxed mechanically from 5 s isometric tetanic contractions at 4°C. As shown in fig.3a, the increased [P_i] returned to the resting level exponentially with the time constant of 18.7 min. Assuming that the internal pH recovers in a single exponential manner after contraction, the time course of pH recovery was best explained by a single exponential with the same time constant (18.7 min) as that for [P_i].

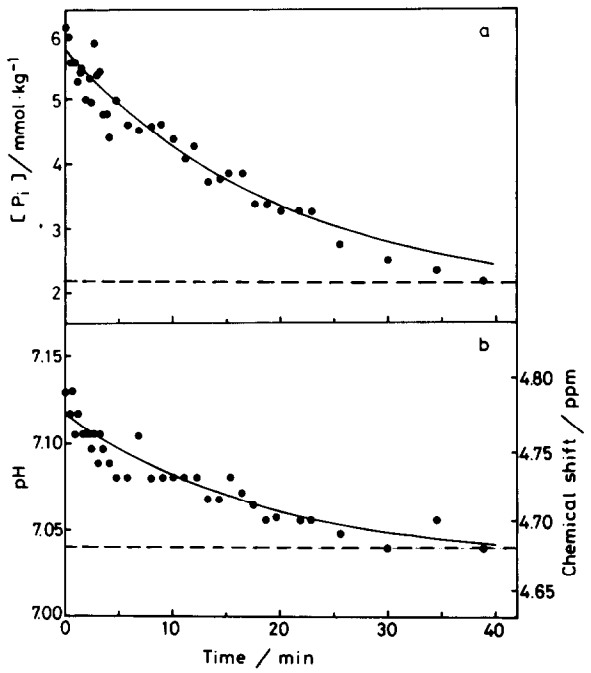

Fig.3. Time course of the changes: (a) in the intracellular [P_i]; (b) in the intracellular pH after 5-s tetanic contractions. Time zero is the time at which the muscles have completely relaxed from a contraction. The curves drawn in solid lines are both exponential with a time constant (τ) of 18.7 min. The resting levels of the intracellular [P_i] and pH are indicated by the dashed lines.

4. DISCUSSION

The intracellular pH of the resting bullfrog sartorius muscles was determined as 7.04 from the chemical shift of P_i, which is in good agreement with results in [2]. The intracellular pH was increased by as much as 0.08 by the 5-s isometric tetanic contraction. The author in [7] observed a slight alkalinization following a contraction at the muscle surface. Authors in [8] showed an increase in intracellular pH of 0.002–0.004 following the stimulation of highly stretched muscle fibers.

The alkalinization of muscles by contraction can be explained as follows. Under a well oxygenated condition, intermediate metabolites such as lactate may not be increased during a short tetanic contraction. If this is the case only the hydrolysis of ATP need be considered. The level of ATP is buffered by PCr through Lohmann reaction. Therefore, the net chemical change is the hydrolysis of PCr into creatine (Cr) and P_i in muscle cells during a contraction as follows:

$$PCr^{2-} + H_2O \longrightarrow Cr^0 + HPO_4^{2-}$$

The second dissociation constant of P_i is 6.88 [5] and hence a part of P_i exists in the $H_2PO_4^-$ form at around a neutral pH:

$$HPO_4^{2-} + H^+ \rightleftarrows H_2PO_4^-$$

At pH 7.08, which is the average value of the intracellular pH just before and after the contraction, 39% of the produced HPO_4^{2-} should be protonated to $H_2PO_4^-$ according to the equation

$$\log([H_2PO_4^-]/[HPO_4^{2-}]) = pK_a - pH$$

Thus the changes in cell composition are expressed by the sum of the above reaction formulas at pH 7.08.

$$PCR^{2-} + H_2O \longrightarrow$$
$$Cr^0 + 0.61HPO_4^{2-} + 0.39H_2PO_4^- - 0.39H^+$$

This indicates that the absorption of protons, or alkalinization, occurs in muscle cells during contraction.

From the present results we can estimate the intracellular buffering power according to the above reaction formula. As shown in fig.3a, the increase in [P_i] is 3.6 mM and thus the amount of protons absorbed is calculated to be 1.4 mM. Since the in-

crease in pH is 0.08, the buffering power is estimated to be 18 mM/pH unit for bullfrog sartorius muscles.

The buffering power estimated here is lower than those (35 mM/pH, 25°C and 44 mM/pH, room temperature) measured for *Rana temporaria* muscles [9,10]. If a significant amount of lactic acid were produced following contraction, the extent of the observed alkalinization (0.08 pH unit) would be less than that expected from the P_i released, due to the acidification by the lactic acid formed. If this were the case the buffering power would be even smaller than 18 mM/pH unit. It is unlikely, therefore, that a significant amount of lactic acid would be produced under the present conditions.

The time course of recovery in the intracellular pH was explained by a single exponential with the same time constant as that for the recovery in the P_i concentration. This is consistent with the assumption that the breakdown of PCr is the only major process occurring in muscles under well oxygenated conditions during short tetanic contractions. The possibility remains, however, that a rapid recovery occurred during the initial 5 min of the recovery period and the level of intracellular pH then returned gradually to the resting level.

Unlike the $[P_i]$, the time course of the recovery of PCr cannot be expressed by a single exponential because of the post-contractile splitting [6]. The PCr level just after the relaxation of contraction is higher than the level expected from a single exponential recovery. This is thought to be due to a time lag between the utilization and consumption of PCr [6]. The present results are consistent with the post-contractile PCr hydrolysis, when the HPO_4^{2-} bound to proteins or membranes is released into the sarcoplasm accompanying a contraction without delay.

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REFERENCES

[1] Radda, G.K. and Seeley, P.J. (1979) Annu. Rev. Physiol. 41, 749–769.
[2] Roos, A. and Boron, W.F. (1981) Physiol. Rev. 61, 296–434.
[3] Bárány, M. and Glonek, T. (1982) Methods Enzymol. 85, 624–676.
[4] Gadian, D.G. (1982) Nuclear Magnetic Resonance and its Applications to Living Systems, Clarendon Press, Oxford.
[5] Dawson, M.J., Gadian, D.G. and Wilkie, D.R. (1977) J. Physiol. 267, 703–735.
[6] Yamada, K. and Tanokura, M. (1983) Jap. J. Physiol. 33, 909–919.
[7] Distèche, A. (1969) Mem. Acad. Roy. Belgique 32, 1–169.
[8] Baylor, S.M., Chandler, W.K. and Marshall, M.W. (1982) J. Physiol. 331, 105–137.
[9] Bolton, T.B. and Baughan-Jones, R.D. (1977) J. Physiol. 270, 801–833.
[10] Curtin, N.A. (1983) J. Muscle Res. Cell Motility 3, 507.