EXCHANGE OF ACTIN-BOUND NUCLEOTIDE IN BRIEF ELECTRICAL STIMULATION OF MUSCLE

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SUMMARY

In frogs injected with tubocurarine and subsequently with ^{14}C-glucose, the specific activity of the actin-bound ADP in the skeletal muscles is low in relation to that of the total ATP of the tissue. Brief electrical stimulation, even a single electrical impulse, brings about a large increase in the specific activity of the bound ADP over that in an unstimulated control. This observation is consistent with a change in the physical state of actin during the contraction of muscle.

INTRODUCTION

In an earlier paper[1] it was shown that in frogs injected subcutaneously with ^{14}C-glucose, radioactive label rapidly appeared in the ADP bound to actin in the skeletal muscles. Experiments with paired muscles gave evidence that contracture with potassium chloride promoted exchange of nucleotide between the myofibrils and their environment. When frogs were curarised before or after injection of ^{14}C-glucose, it was found that the consequent inhibition of postural activity also inhibited exchange of nucleotide.

The experiments described here were intended to establish whether a turnover of bound nucleotide could be shown to result from brief electrical excitation of the muscles. An exchange due to a single electrical impulse might clearly be regarded as germane to the physiological process of contraction.

MATERIALS AND METHODS

Frogs of the species Rana temporaria were employed. They were usually stored at 4°C for several days before an experiment, although no special care was taken to achieve a reproducible physiological state.

[U-^{14}C] glucose was obtained from the Radiochemical Centre, Amersham. The enzymes used in the nucleotide assays (hexokinase, glucose-6-phosphate dehydrogenase, pyruvate kinase), and phosphoenolpyruvate were supplied by

Boehringer, the co-factors (ATP, NADP, NADH,) by Sigma. All other reagents were of analytical quality when this was obtainable.

Nucleotide assays were made with a Locarte single-sided fluorimeter, Mark 4, with Locarte filters LF/2 (340-380 nm) as primary filter and LF/5 (440 nm) as photomultiplier cut-off.

Radioactive counting was carried out with a Panax dekatron scaler, type D657C, together with a Panax scintillation counter, Type SCA.

Electrical stimulation of muscles was effected with a Palmer square-wave generator through platinum electrodes set 5 mm apart in a plastic mount.

The Ringer's solution had the composition: 6.5 g NaCl, 0.14 g KCl, 0.12 g $CaCl_2$, 0.2 g $NaHCO_3$ per 1.

The experiments were performed as described in the earlier paper[1] except for the mode of excitation of the muscles.

(+)-tubocurarine (20 µg per g body weight) was injected subcutaneously and the frogs were left at room temperature for 1 hr. 25 µCi of [U-^{14}C] glucose was then administered subcutaneously into the abdomen.

After the prescribed incubation period, the animals were pithed and the muscles dissected out quickly. In some experiments with sartorii, the test muscle was stimulated, after removal of the control, with its pelvic attachment still intact and with the peripheral end held firmly in forceps. In the remainder of the electrical experiments, the muscles were pinned to a cork board as near the ends as possible; with the rectus abdominis, the xiphisternum was used as a point of anchorage. Contractions were hence approximately isometric. Control and test muscles were manipulated in similar fashion. The selection of the control muscle from a pair was random.

The muscles were minced rapidly with fine scissors under Ringer's solution. About three-quarters of the material was used for isolation of actin-bound ADP by the following procedure:

The muscle was extracted for 20 min. in 0.05M-Na_2CO_3/0.05M-$NaHCO_3$ and washed ten times with ice-cold distilled water over 2 hr. The residue was treated for 10 min. with 3 ml ice-cold 10% (w/v) trichloroacetic acid. The solution was centrifuged and freed from trichloroacetic acid by shaking ten times with cold ether. It was then poured into a 15 ml siliconed tube and freeze-dried. The residue, dissolved in 10 µl water was used for chromatographic separation of ADP.

The smaller portion of muscle, used for isolation of ATP, was extracted with 3 ml 10% (w/v) trichloroacetic acid for 10 min. The solution was shaken ten times with cold ether and brought to pH 8.2 with dilute NaOH. 0.3 ml 10% (w/v) barium acetate was added. After 16 hr. the precipitate, including BaATP, was centrifuged and dissolved in 10 µl 2N formic acid for chromatography.

The nucleotides were isolated by chromatography on Whatman No. 1 paper by the method of Krebs & Hems[2]. The chromatograms were dried at room temperature and the spots bearing the nucleotides, detected with a Hanovia "Chromatolite" UV lamp, were extracted with 1 ml water. The solutions were stored at $4^{\circ}C$ and used for assay and radioactive counting within a few hours.

ADP and ATP were assayed in 0.3 ml aliquots of the solution in a total volume of 1.5 ml by the fluorometric procedure of Estabrook et al.[3]. Triethanolamine-HCl buffer, pH 7.4, was used for ATP determination and Na-K phosphate byffer, pH 7.0, for ADP determination.

Radioactivity was measured by the emulsion technique of Patterson & Green[4]. The scaler was adjusted to give 85% efficiency with a sealed ^{14}C-source. An aliquot (0.5 ml) of nucleotide solution was pipetted into 10 ml phosphor (toluene : Triton X 100, 2:1 v/v, containing 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-2-(4-methyl-5-phenyloxazolyl-benzene) at $4^{\circ}C$ and allowed to equilibrate in the dark at $4^{\circ}C$ for 10 min. Blanks were prepared in the same phosphor using water in place of nucleotide solution.

TABLE 1

Effect of stimulation with single electrical impulse on specific activities of total ATP and actin-bound ADP in rectus abdominis of curarised frogs labelled with ^{14}C-glucose.

Experimental procedure as in text. Stimulus : 15 V, 0.5 msec.

Specific activity (counts/μmole/min.)			
Total ATP		Bound ADP	
Control	Contracted	Control	Contracted
1000	1630	110	430
3600	4900	290	540
530	1270	200	250
280	390	110	460
2600	4800	290	1390
2000	3600	150	1640
2470	4870	260	1650
1960	4490	390	1130

RESULTS

The results of experiments with the rectus abdominis are shown in Table 1. Excitation with a single electrical impulse resulted in an increase in the specific activity of total ATP, and in an increase, often disproportionate, in that of actin-bound ADP. The outcome of this series and one where the sartorius was brought into a 5-second tetanus, are summarised in Table 2, together with that of analogous experiments in potassium contracture taken largely from the earlier paper[1]. Comparison is facilitated by referring all specific activities to a value of 1000 for that of total ATP in the control muscle. All results are included, with the exception of two in the tetanus group where the estimations of total ATP were suspect. In every case, including the two omissions, stimulation resulted in an increase in the specific activity of the protein-bound ADP.

TABLE 2

Effect of stimulation on specific activities of total ATP and actin-bound ADP in skeletal muscles of curarised frogs labelled with ^{14}C-glucose.

Experimental procedure as in text. For KCl contracture, see ref. 1. Electrical stimulus : 15 V, 0.5 msec. (50 pulses/sec. for tetanus).

		Relative specific activities			
	No. of Expts.	Total ATP Control	Total ATP Stimulated	Bound ADP Control	Bound ADP Stimulated
Sartorius - 5 sec. tetanus	5	1000	3970 ± 2090	230 ± 410	1870 ± 490
Rectus abdominis - single twitch	8	1000	1830 ± 380	180 ± 130	660 ± 440
Rectus abdominis - KCl contracture	5	1000	2380 ± 700	-70 ± 150	1370 ± 790

DISCUSSION

Despite the errors inherent in experiments of this type, the results lend support to the view that activation of muscle is followed by exchange of nucleotide between the myofibrils and their environment. Exchange after comminution of the muscle seems relatively unimportant, since this would hardly be inhibited by tubocurarine. In phasic muscles of uncurarised animals, the specific activity of bound ADP may rapidly overtake that of total ATP.[1] With curarised animals, however, the bound ADP usually acquires only a small degree of labelling, an effect evidently due to blocking of the motor end plates by the drug.

The exchange can be assessed only if it be supposed that the specific activity of the total ATP is not widely different from that of the nucleotide pool in exchange with the myofibrils. The results of our experiments with potassium contracture[1] tend to justify this assumption. On this basis, an isometric twitch would seem to be accompanied by an exchange of some 25% of the bound nucleotide, a 5-second tetanus by one of 40% or more. These

estimates, however, could be too modest, since the synthesis of ATP from labelled precursors, stimulated by excitation of the tissue, probably continues at a higher level after the conclusion of activity. There is every reason to believe that all the actin-bound nucleotide becomes exchangeable in the active state.

The experiments of Moos et al.,[5] Moos & Eisenberg[6], and Appenheimer et al.[7] on actomyosin, extracted myofibrils and isolated F-actin, led the authors to believe that the nucleotide exchange in these systems occurs only at sites of disorganisation in the quaternary structure of F-actin. The sluggish exchange in living muscle reported by Martonosi et al.[8] would reflect processes of repair made necessary by stresses imposed on the thin filaments in contraction. Our results suggest that this explanation is scarcely tenable for intact tissue. It seems more reasonable to suppose that the exchange of bound nucleotide results from a configurational change in actin which is an essential feature of contraction.[9] The X-ray diffraction data for active muscles obtained by Huxley and his collaborators[10,11] seem not to exclude such a change.

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