ADENOSINE TRIPHOSPHATE BREAKDOWN DURING A SINGLE ISOTONIC TWITCH OF FROG SARTORIUS MUSCLE†

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Recent work has shown that 1-fluoro 2,4 dinitro-benzene (FDNB) can inhibit creatine phosphoryltransferase in situ in isolated frog muscle. This made possible the first direct demonstration of the breakdown of amounts of adenosine triphosphate (ATP) quantitatively sufficient to account for the work done in a single smooth tetanic contraction of electrically stimulated frog rectus abdominis muscle. (Cain and Davies 1962) However, this muscle is slow and does not show classical twitch responses, so experiments have now been carried out with the intensively studied sartorius muscle which is fast and can perform normal twitches as well as tetanic contractions.

Methods

The methods and analytical procedures were largely those already described by Cain and Davies (1962). The sartorius muscles of female frogs (Rana pipiens) were dissected, weighed and allowed to rest at room temperature for at least one hour in a physiological bicarbonate saline solution gassed with 5% CO_2 in 95% O_2. They were then transferred to a similar solution, gassed with 5% CO_2 in 95% N_2 and containing 3.8×10^{-4}M FDNB at 0°C for 40 min. and mounted on an isotonic lever at rest length. They were then frozen rapidly in a 1:1 mixture of Freon 12 and 13 (CF_2Cl_2 and CF_3Cl, -170°C) either without further treatment (control) or at various times during a

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single twitch following a supramaximal electrical stimulation of 0.4 milliseconds duration.

The instant when the twitch was interrupted by freezing was controlled by a simple switching device. The control muscles were selected alternately from the left and right leg and remained at rest length while being frozen. The frozen muscles were cut down and rapidly transferred while frozen to stainless steel centrifuge tubes at -196°C. Each muscle was pulverized at this temperature with a cold, steel ball on a handle and ground into 0.2 ml 0.5 M $HClO_4$ to form a smooth powder. The powdered muscle plus perchloric acid was stored in liquid nitrogen. Extracts were thawed in pairs, 1.0 ml of 0.5 M $HClO_4$ was added to each extract and the tube transferred to a water bath at 35°C. The contents were stirred for 4 min., spun in the high speed head (#296) of an International Centrifuge, then 1.0 ml of the supernate was rapidly removed and added to 0.45 ml of a solution containing 0.5 m-mole KOH + 0.15 m-mole triethanolamine buffer, pH 7.5. The solution was refrozen and stored at -78°C until ready for analysis. After rethawing, the precipitate of $KClO_4$ was allowed to settle at 0°C. ATP was measured fluorimetrically in duplicate as previously described. In several experiments, the ATP content of control and contracted muscle was also measured by the use of the specific firefly luminescence technique and a photomultiplier coupled to a recorder.

Results

Whereas isolated sartorius muscles can twitch very many times at 0°C, these muscles after treatment with FDNB contained normal amounts of ATP cf. (Carlson and Siger 1959; Mommaerts 1955) but could do only 8 quite typical twitches. However, even in a short tetanus they did not break down phosphorylcreatine (PCr). The work done during a single isotonic twitch of these sartorius muscles was only about one seventh of the 125 gm-cm of work per gram of muscle performed during the previous experiments on contractions of the rectus abdominis muscle (Cain, Infante and Davies, 1962). Thus, the expected change in the ATP was small. Tables 1 and 2 present every completed experiment we have carried out. No results have been rejected. The variation

in individual experiments is probably due to random differences between the two members of each pair of sartorii from the frog. It is clear from Table 1 that ATP is actually broken down during the rising phase of a single twitch. Moreover, Table 2 shows that the muscles which relaxed somewhat before being frozen broke down even more ATP ($0.05 > P > 0.02$).

TABLE 1

Changes in adenosine triphosphate in muscles frozen at the peak of the rising phase of a single isotonic twitch at 0°C of frog sartorius muscles pretreated with fluorodinitrobenzene

Muscle Pair	Control ATP (μmole/gm)	Twitch ATP (μmole/gm)	ΔATP (μmole/gm)	External Work (gm-cm/gm)	Percent Shortening $\frac{l_o - l_s}{l_o} \times 100$	Percent Relaxation $\frac{l_r - l_s}{l_o} \times 100$	Time (Sec)
1	2.55	2.39	-0.16	16.4	9.7	0.0	0.09
2	3.38	3.25	-0.13	16.7	9.7	0.0	0.09
3	2.55	2.53	-0.02	16.3	5.9	0.0	0.17
4	2.82	2.58	-0.24	18.0	7.7	0.0	0.09
5	3.25	3.12	-0.13	9.9	3.1	0.0	0.09
6	2.93	2.57	-0.36	8.7	3.9	0.0	0.10
7	3.38	3.20	-0.18	25.0	10.8	0.0	0.17
8	2.16	2.01	-0.15	14.1	6.0	0.0	0.17
9	3.16	2.98	-0.18	25.5	14.3	0.0	0.26
10	2.51	2.40	-0.11	16.6	10.9	0.0	0.17
11	1.90	2.09	+0.19	20.3	13.1	0.0	0.26
12	2.90	2.40	-0.50	21.0	11.2	0.0	0.09
13	3.56	2.64	-0.92	17.5	8.8	0.0	0.31
Mean	2.85 ± 0.14	2.63 ± 0.10	-0.22 ± 0.07	17.4 ± 1.3	8.9 ± 0.9	0.0	0.16 ± 0.02

All results are given with the standard error of their means; l_o, l_s and l_r are respectively the initial (rest) length, the length at the peak of shortening and the length following partial relaxation. Time refers to the approximate duration of the twitch.

Discussion

The actual gross breakdown of ATP in these experiments may well be greater than that observed because of a possible resynthesis of ATP from adenosine diphosphate by myokinase during the contraction (cf. Cain and Davies,

1962). However, the observed breakdown for the external work done given in Table 1 fits well with the relation between work done and PCr breakdown found previously with frog rectus abdominis muscles (Davies, Cain, Infante, Klaupiks and Eaton, 1962; Cain, Infante and Davies, 1962). The extra breakdown during relaxation was unexpected both because of the absence of a biochemical relaxation heat (Hill, 1953) and because no change in inorganic phosphate was found during relaxation of the rectus abdominis muscles (Cain, 1960; Cain and Davies, 1960).

TABLE 2

Changes in adenosine triphosphate in muscles frozen about one half way through the relaxation phase of a single isotonic twitch at 0°C of frog sartorius muscles pretreated with fluorodinitrobenzene

Muscle Pair	Control ATP (μmole/gm)	Twitch ATP (μmole/gm)	ΔATP (μmole/gm)	External Work (gm-cm/gm)	Percent Shortening $\frac{l_o-l_s}{l_o}$ x 100	Percent Relaxation $\frac{l_r-l_s}{l_o}$ x100	Time (sec)
1	2.15	2.22	+0.07	14.3	9.4	5.9	0.26
2	1.49	0.92	-0.57	14.2	10.0	6.2	0.26
3	2.31	2.19	-0.12	15.3	12.9	1.1	0.26
4	3.05	1.99	-1.06	15.4	11.3	6.0	0.31
5	3.14	2.80	-0.34	15.8	7.8	2.3	0.17
6	3.28	2.74	-0.54	18.1	11.7	3.8	0.17
7	2.75	2.30	-0.45	21.3	13.5	11.1	0.26
8	3.66	3.30	-0.36	21.7	7.0	4.9	0.26
9	3.72	3.41	-0.31	11.8	5.0	0.3	0.09
10	3.32	2.90	-0.42	10.7	2.6	1.4	0.17
11	3.57	3.04	-0.53	22.9	7.0	5.4	0.20
12	3.35	2.92	-0.43	22.0	9.9	7.0	0.26
13	3.15	2.69	-0.46	15.1	5.6	4.8	0.17
Mean	3.00 ± 0.18	2.57 ± 0.18	-0.43 ± 0.07	16.8 ± 1.0	8.8 ± 0.9	4.6 ± 0.8	0.22 ± 0.02

All results are given with the standard error of their means; l_o, l_s and l_r are respectively the initial (rest) length, the length at the peak of shortening and the length following partial relaxation. Time refers to the approximate duration of the twitch.

This difference may be explained as follows: on the basis of measurements during the first 1 or 2 seconds the changes of high-energy phosphate on stimulation of the slow rectus abdominis are very small (Cain, Infante and Davies, 1962) and are less than 0.01 µmole PCr/stimulation/gm. Recently Distèche measured the amount of PCr associated with activation in frog sartorius muscles in hypertonic solutions and found a value of about 0.2 µmole PCr/activation. He measured a series of activations and extrapolated backwards to get this number so the precise time when the PCr was used was not known. It is interesting that the differences between the results in Table 1 and 2 is about the difference expected per activation. If it is assumed that the activation of muscle contraction is caused by an electrically-stimulated release of calcium into the cytoplasm, which then causes cross-link formation and tension development with release of heat, it follows that work must be done later to pump this calcium back into the endoplasmic reticulum (Molnar and Lorand, 1962; Hasselbach and Makinose, 1962). In our experiments this process would be observed immediately after the rising phase of the contraction in the fast sartorius muscle, but would be delayed in the slow rectus abdominis muscle. Thus an extra amount of high-energy phosphate would be needed for the reversal of the tension-generating mechanism and this may account for the extra breakdown of ATP after the work has been done.

In summary, a breakdown of ATP has been observed during the rising phase of a single isotonic twitch of frog sartorius muscle. A delayed breakdown of ATP also occurs after the mechanical work is completed which may be associated with the recovery after activation during the continuance of the active state.

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