ABSENCE OF RESPONSE TO ADENOSINETRIPHOSPHATE IN A GLYCERINATED UNSTRIATED MUSCLE

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The enzymatic splitting of adenosinetriphosphate, providing the free energy of hydrolysis for immediate utilisation, has been considered the primary event in muscular contraction. Fleckenstein *et al.* (1954 *a*, *b*) found no evidence for ATP, ADP, or phosphocreatine changes after 1 to 2 second tetanus of the frog's rectus abdominis at 0° C. They reported of an increase in inorganic phosphate and of an unidentified fraction, suggesting the possibility of another source of energy-rich phosphate. Mommaerts (1954) found no evidence for the fission of either ATP or phosphocreatine during single twitches of turtle muscle; according to him " the possibility of a new 'Revolution in Muscle Physiology' is clearly discernible ". Professor V. A. Engelhardt has spoken that this was not a revolution but only a change in cabinet. Dixon and Sacks (1958) using radio-active tracers, also found no evidence for ATP breakdown during contraction.

In attempting to study the effect of ATP on unstriated muscle of frog's stomach, it was noted that after the usual treatment with 50 per cent. glycerine and refrigeration, it does not respond to ATP with any contraction. Since this muscle shows the usual physiological responses found in muscle, the models of this muscle should respond to ATP like other muscle models, if this substance was the primary source of energy for muscular contraction. In the present research, therefore, a detailed investigation of the effect of ATP on the models prepared from this muscle has been undertaken.

Experimental

These experiments were performed on transverse pieces of the stomach muscle of the frog, *Rana tigrina*. After dissection, they were immersed in 50 per cent. glycerine and stored in a refrigerator at — 15 to — 20° C., for variable periods ranging from 24 hours to 6 months. Some muscles were stored at 0° C. The fluid was exchanged twice for fresh, precooled 50 per cent. glycerol, at daily intervals before storage. Before use the fibres were

placed in 15 per cent. glycerol at 0° C. for one hour; but 15 per cent glycerol causes marked lengthening of the muscle, and so is not suitable.

The effect of ATP was tested on loaded as well as unloaded pieces; the latter were used to test for active relaxation, if any. The experiments were performed at room temperature, 30° C.

RESULTS

As reported previously (Singh and Acharya, 1958), the effect of ions on frog's stomach muscle which has been refrigerated in 50 per cent. glycerine is different from that on striated muscle. Potassium chloride causes contraction of glycerine soaked striated muscle (Bozler, 1951). Similarly treated frog's stomach muscle from the cardiac end actively relaxes by about 60 per cent. in isotonic (0·112 *M*) potassium chloride. Calcium or magnesium (0·01 *M*) antagonises this action, so that the relaxation is reduced to 40 per cent.

In the present experiments, pieces of muscle were removed from the refrigerator and glycerine was removed by washing with potassium chloride solution, with or without magnesium. The treatment with potassium chloride solution continued till a constant length was reached in the freely floating condition. To test the effect of ATP on loaded muscles, they were suspended in a bath; to test for active relaxations, the preliminary solution was changed for that containing ATP with the muscle freely floating in a Petri-dish.

Effect of ATP on contraction.—The effect of the sodium salt of ATP in concentrations of 1·4, 1, 0·5, 0·25, 0·1, and 0·05 per cent. was tested. The muscles were treated beforehand with 0·112 *M* potassium chloride solution alone, or magnesium chloride in concentrations of 0·001 to 0·01 *M* was also added. In some experiments, calcium chloride in concentrations of 0·05 and 0·01 *M* was also included with or without magnesium in the potassium chloride solution. The ATP was dissolved in the preliminary solution with which the muscle was treated, but in concentration of 1·5 per cent. it was also used dissolved in distilled water. In over 50 experiments with loaded muscles, no contraction was ever observed. The muscles were stored in the refrigerator for 24–48 hours, 10 days, 1 month, 3 months and 6 months. In over 100 experiments with unloaded muscles, stored for 24–48 hours, 20 experiments with muscles stored for 10 days, 20 experiments with muscles for 1 month, 20 experiments with muscles stored for 3 months and 20 experiments with muscles stored for 6 months,

no contraction was ever observed. The addition of CP to ATP solutions made no difference.

Effect of ATP on active relaxation.—In the above 100 experiments with unloaded muscles, 12 pieces relaxed activity by about 10–40 per cent. (Table I). In muscles stored for longer time, no active relaxation has been even observed. As the positive results are few, these findings are of doubtful value. But it is also possible that the failure to get active relaxation by ATP in the great majority of experiments was due to preliminary active relaxation by potassium chloride, so that ATP was unable to cause any further relaxation.

TABLE I

Frog's stomach muscle. Active relaxation produced by ATP. Muscle previously soaked in 50 per cent. glycerine for 24–48 hours and then washed

Number of Muscle	Active relaxation per cent. of initial length	Solution
1	24	0·5 per cent. ATP in KCl
2	40	Do.
3	33	Do.
4	28	Do.
5	23	Do.
6	44	Do.
7	10	0·01 *M* $MgCl_2$ and 0·5 per cent. ATP in KCl
8	10	Do.
9	12	0·01 *M* $MgCl_2$ and 0·1 per cent. ATP in KCl
10	15	Do.
11	12	Do.
12	15	0·01 *M* $MgCl_2$ and 0·05 per cent. ATP in KCl

DISCUSSION

It appears that ATP does not cause contraction of glycerinated unstriated muscle from the stomach of the frog *Rana tigrina*. The living muscle behaves like any other muscle, so that these experiments throw doubt on the role of ATP as the primary source of energy in muscular contraction.

There is some possibility of active relaxation being produced by ATP. In this muscle, relaxation is attended with increase in oxygen consumption (Rao and Singh, 1940), and lactic acid production which is abolished by iodoacetic acid and cyanide along with active relaxation (Bharadwaj and Singh, 1951). Increased production of lactic acid during relaxation has been found by Mohme-Lundholm (1953, 1956); adrenaline, noradrenaline, isopropylnoradrenaline, and ephedrine have both a relaxing and lactic acid-forming effect. The degree of relaxation tends to run parallel with the increase in lactic acid concentration. Excess of calcium ions inhibit both the relaxing and the lactic acid-forming effect of adrenaline.

There is thus no doubt that there is breakdown of glycogen during relaxation of unstriated muscle, and as breakdown of glycogen is known to be preceded by fission of phosphocreatine and ATP, the production of active relaxation by ATP is theoretically possible; and so if the positive results in actual experimentation are few, nevertheless they are significant.

In unstriated muscle, ATP or some other substance might be acting on two different contractile mechanisms. There is evidence that unstriated muscle contains two contractile mechanisms, one of which relaxes actively and the other possively (Singh and Acharya, 1958).

The presence of two contractile mechanisms in unstriated muscle is shown by physiological experiments. Thus it shows two kinds of tone; one declines if the muscle is asphyxiated, but the other is unaffected by such a treatment; it may even increase (Singh, 1949). This shows that the former tone requires energy for its maintainence, but the latter is maintained without such expenditure of energy; it may last till the death of the muscle. The phasic response disappears if the muscle is asphyxiated, so that it would appear to be produced by the first contractile mechanism. This is shown by another experiment. In the dog's stomach muscle, it is possible to destroy tone by quick stretch, without affecting the phasic response, and the tone which is thus destroyed is that which is resistant to asphyxia (Singh and Singh, 1949). In the frog's stomach muscle, tone which is resistant to asphyxia, can be similarly destroyed, without affecting the phasic response (Singh and Singh, 1950), but if the tone, which is sensitive to asphyxia, is

destroyed by quick stretch, then the phasic response is also damaged (1957 *a*, *b*). This clearly indicates that there is one contractile mechanism which is responsible both for the phasic response and the tone which is diminished by asphyxia, and the other mechanism which is responsible for asphyxia-resistant tone. There is considerable other evidence for the existence of two systems in muscle (*see* Gelfan, 1958) for references.

Summary and Conclusions

1. Glycerinated frog's stomach muscle from *Rana tigrina* does not respond to ATP with contraction. As this muscle responds physiologically like other muscles, this throws doubt on the accepted role of ATP in muscular contraction.

2. In a few glycerinated muscles ATP has produced active relaxation. As the number of positive results is small, they are of doubtful value. But theoretically such a possibility exists as increased metabolism definitely occurs during relaxation.

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