Histochemical studies on adenyl cyclase activity in normal and dystrophic skeletal muscle of mice*

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In murine dystrophy the content of triglycerides and free fatty acids (FFA) in muscle tissue is much greater than in normal mice (Shull and Alfin-Slater, 1958). It has been suggested that the FFA derived from several sources get accumulated in the dystrophic muscle because they are not utilized in the normal way for energy release and that the accumulated FFA get re-esterified into triglycerides (Susheela *et al.*, 1968).

Recent studies (Angel *et al.*, 1971) on epididymal fat cells have shown that increased levels of FFA reduce the content of adenosine triphosphate (ATP)—an essential substrate for the production of adenosine 3′,5′-monophosphate (cyclic 3′,5′-AMP). In view of the known dependence of energy releasing mechanisms in the cell on cyclic 3′,5′-AMP, the present studies were carried out to determine whether the increased amounts of FFA in the dystrophic murine muscle also adversely affect the production of cyclic 3′,5′-AMP.

These investigations by histochemical methods were carried out by studying the distribution of an enzyme, adenyl cyclase (AC), responsible for the production of cyclic 3′,5′-AMP. The histochemical technique for localizing AC activity was developed in our laboratory and the details of this technique and its specificity for localizing AC will be communicated elsewhere.

MATERIAL AND METHODS

Dystrophic mice and non-dystrophic litter-mates of the Bar Harbor Strain 129 as well as normal mice (locally available strain) were used in the present studies.

Histochemical localization of adenyl cyclase activity

The animals were anaesthetized by an intraperitoneal injection of 3% solution of chloral hydrate given at a dose of 1 ml/100 g body weight and the animals were perfused with 1% glutaraldehyde in 0.05 M solution of sodium cacodylate buffer (pH 7.4) containing 4.5%

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dextrose. Soleus (Type I) and gastrocnemius (Type II) muscles and subcutaneous fat were dissected out, sliced into tiny pieces of about 2 mm length and 1 mm width and fixed in the same fluid for an additional period of 3 to 4 hr. The tissues were washed for about 12 to 15 hr in several changes (5 to 6 changes) of sodium cacodylate buffer solution containing 4.5% dextrose and then rinsed in distilled water. All the solutions were used at room temperature (20° C). Different pieces of tissues were then separately incubated in the following media for a period of 3 hr at room temperature.

Medium 1 contained 0.1 ml each of tris maleate buffer (80 mM, pH 7.4), 8% dextrose, 2 mM of theophylline, 4 mM of magnesium sulphate, 4.8 mM of lead nitrate and 0.5 mM of ATP.

Medium 2 was same as medium 1 but contained 0.1 ml of 4×10^{-6} M isoproterenol, an AC activator.

Medium 3 was same as medium 1 but without the substrate ATP.

Medium 4 was same as medium 1 but contained 5 mM of p-chloromercuribenzoic acid, an ATPase inhibitor.

Following incubation all the tissues were washed in tris maleate buffer (pH 7.4) containing 8% dextrose and then treated with a 1% aqueous solution of yellow ammonium sulphide.

The pieces of tissues were dehydrated, cleared, embedded in paraffin wax, sectioned 10 to 12 μ and mounted on glass slides. The sections were dewaxed and mounted under cover glass in DPX and viewed under an ordinary light microscope.

Other tissues such as hypophysis, pineal, thyroid and adrenal were also taken from the dystrophic mice to test the validity of the results obtained for the dystrophic muscle.

For assessing the cellular damage, pieces of dystrophic muscle were processed, sectioned and stained with haematoxylin-eosin.

RESULTS

Histochemical demonstration of adenyl cyclase

The sites of AC activity can be demonstrated as brownish black precipitate only in those tissues incubated in media 1 and 2 but not in tissues incubated in media 3 and 4. The intensity of reaction was, however, more in tissues incubated in medium 2 which contained isoproterenol (AC activator).

Adenyl cyclase in non-dystrophic mice

In the Type I and Type II fibres, AC activity was localized in the sarcolemma and in small granules, probably mitochondria, in the sarcoplasm. The AC activity was different between Type I and Type II fibres; the Type II fibres showed a considerably higher concentration of the lead sulphide granules in contrast to Type I fibres (Figs. 1 and 2). Occasionally motor nerves innervating the muscle fibres could be seen, and such nerve fibres showed an intense reaction (Fig. 1, insert).

In adipose tissue the AC activity could be demonstrated only in certain parts of the plasma membrane; the other parts did not show any reaction (Fig. 3).

Adenyl cyclase in dystrophic mice

In marked contrast to the controls, AC activity could not be demonstrated in Type I and Type II muscle fibres as well as in the adipose tissue of dystrophic mice. Histological examination of the muscle tissue showed that it had reached an advanced stage of dystrophy and that severe cellular damage had occurred (Figs. 4 and 5).

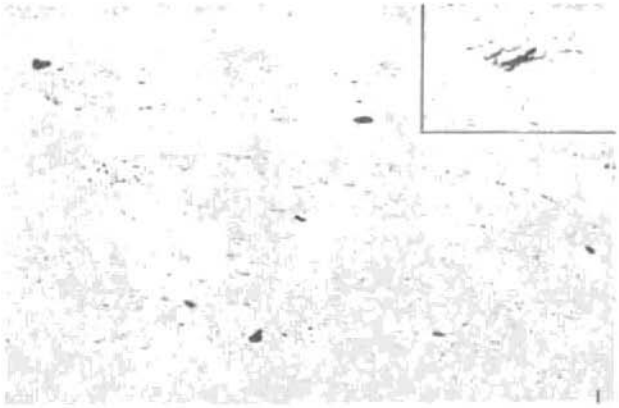

Fig. 1 Longitudinal section of the soleus muscle from a non-dystrophic mouse. The black precipitates indicate the sites of AC activity. Insert shows an intense enzymic reaction in the nerve fibres. × 650; Reduced for reproduction 50%.

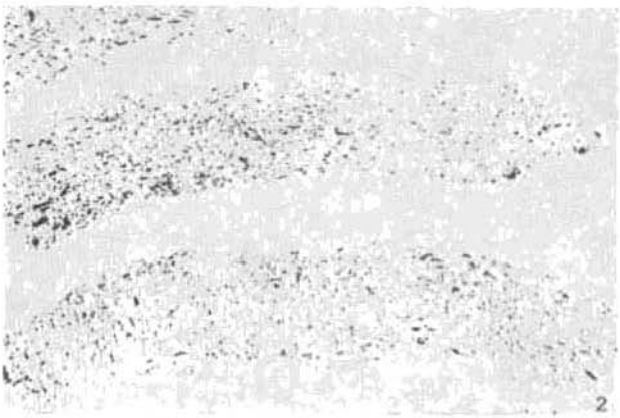

Fig. 2 Gastrocnemius muscle from a non-dystrophic mouse showing AC activity. Note the greater amount of AC activity in contrast to that shown in Fig. 1. × 650; Reduced for reproduction 50%.

The presence of AC activity in other tissues of dystrophic mice, such as the hypophysis, pineal, thyroid and adrenal could, however, be clearly demonstrated.

DISCUSSION

The difference in the AC activity between Type I and Type II fibres must be considered in context with the intrinsic differences between the two types of fibres. The Type I is a lipolytic fibre and Type II is a glycolytic fibre. Cyclic 3′,5′-AMP, in Type I fibre probably has a single role in the conversion of inactive lipase to active lipase, while in the Type II fibres cyclic 3′,5′-AMP has an additional role in activating phosphorylase. It is therefore likely that greater activity of AC found in Type II fibre reflects the greater amount of cyclic 3′,5′-AMP present in this than in Type I fibre.

The absence of AC activity certainly indicates that the levels of the enzyme in both Type I

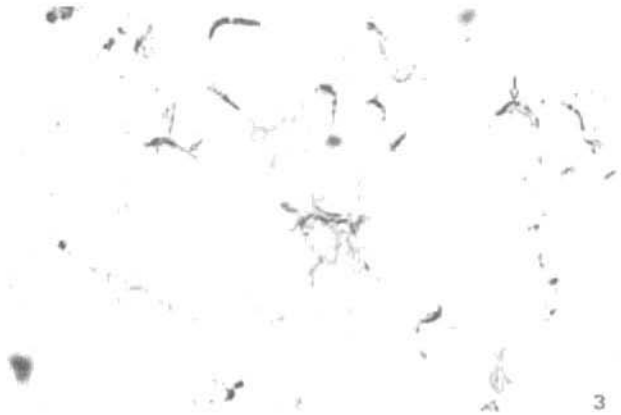

Fig. 3 Photomicrograph of adipose tissue treated for AC activity. Note the enzymic reaction present in certain regions of the fat cell membranes (arrows). ×650; Reduced for reproduction 50%.

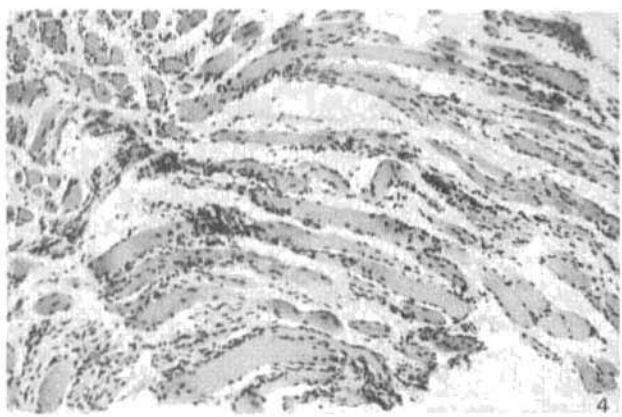

Fig. 4 Soleus muscle; obtained from dystrophic mice revealing the extent of cellular damage. Haematoxylin-eosin. ×160; Reduced for reproduction 50%.

and II muscle fibres of dystrophic mice are either too low to be detected by the method used in this study or they are absent. The possible reason for the low level or absence of AC in the dystrophic muscle may well be due to the high level of FFA. It might be pertinent to refer to the inhibitory effects of high levels of FFA, in fat cells, on the intracellular contents of ATP—a substrate for AC (Angel *et al.*, 1971); high levels of FFA may have a similar effect on the intracellular content of ATP and its enzyme AC in dystrophic muscle.

Since AC has a direct bearing on the formation of cyclic 3′,5′-AMP, the absence of AC in dystrophic muscle would result either in the absence of cyclic 3′,5′-AMP, or it being present in very small amounts. The low level or absence of cyclic 3′,5′-AMP would hamper the activation of lipase and thus result in the non-conversion of triglycerides into FFA. It has already been suggested that the FFA derived through other metabolic pathways would also get re-esterified into triglycerides (Susheela *et al.*, 1968). Thus, the amount of depot fat (mostly triglycerides), would progressively increase in the dystrophic muscle with the advance of the disease.

Fig. 5 Gastrocnemius muscle; obtained from dystrophic mice revealing the extent of cellular damage. Haematoxylin-eosin. ×160; Reduced for reproduction 50%.

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