THE POSSIBLE CELLULAR MECHANISM OF 2,4-DICHLOROPHENOXYACETATE-INDUCED MYOPATHY

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1. Introduction

Chronic treatment with 2,4-dichlorophenoxyacetate (2,4-D) results in a severe myopathy, accompanied by significant biochemical changes, in muscle [1]. The acute effect of 2,4-D is to cause the mitochondria of dystrophic muscle to be swollen and increased in number [2]. In view of the important role of calcium in the regulation of muscle contraction, it seemed of interest to localize calcium in dystrophic muscle.

2. Materials and methods

Adult rats of both sexes were treated with 2,4-D (50 mg/kg body wt/day, i.p.). Muscles of different metabolic type were removed three weeks after the commencement of the treatment. Their fibre-type was determined by staining with diaminobenzidine (DAB), which visualizes the myoglobin-bound peroxidase activity [3]. As the majority of fibres in the semimembranous muscle was not stained, it was considered to be white muscle, whereas the soleus muscle was found by DAB staining to consist mainly of red fibres. The potassium pyroantimonate precipitation technique [4–6] was used to localize calcium in the muscles. Both normal and 2,4-D-treated muscles were removed at their resting length during ether anaesthesia. Samples, tied to wooden sticks, were immersed immediately after removal, in the following solution: 2% potassium pyroantimonate (Fluka) and 2.5% glutaraldehyde in 0.2 M Tris–HCl buffer, pH 7.5, at 4°C for 1 h. After washing in the same buffer, the specimens were fixed in a solution containing 2% potassium pyroantimonate and 1% OsO_4 buffered with 0.2 M Tris–HCl, pH 7.5, for 1 h. Specimens were then dehydrated through graded alcohol solutions and embedded in Durcupan (Fluka). Sections of gold interference colour were cut on a Porter-Blum ultratome, mounted on Formvar-coated copper grids (150 mesh) and covered with carbon. Half the sections were left unstained for energy-dispersive X-ray microanalysis to determine the pyroantimonate precipitate content. The other half were stained on the grids with uranyl acetate and lead citrate and viewed in a Jeol 100B electron microscope.

3. Results and discussion

Figure 1 shows pyroantimonate deposits, 400–750 Å diameter, confined to the sarcoplasmic reticulum of untreated white muscle. The deposits are arranged in a regular manner outside the myofibrils, between the A-I junction, the site of the triads of mammalian muscle [7]. Figure 2 shows a detail of contracted fibres from control white muscle. Signs of spontaneous contractions were only occasionally observed in our material. In contracted muscles, the pyroantimonate precipitates were confined almost exclusively to the sarcoplasmic reticulum, but outside of the vesicles.

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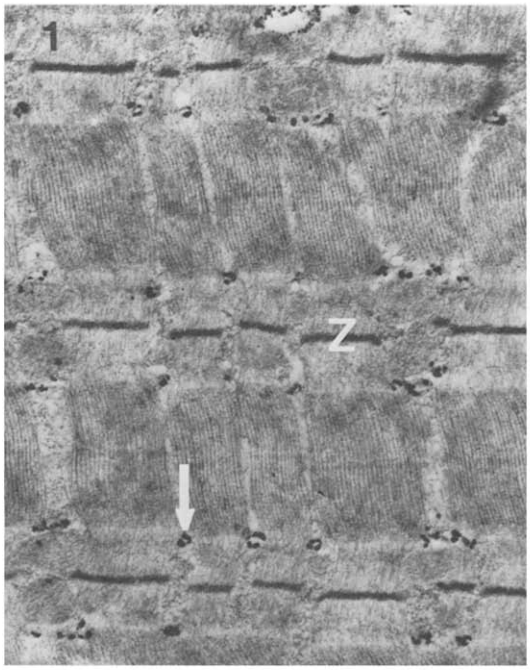

Fig.1. Localization of the pyroantimonate reaction product in control muscle. Precipitates (arrow) are confined to the triads. Z lines are indicated by Z. × 18 900.

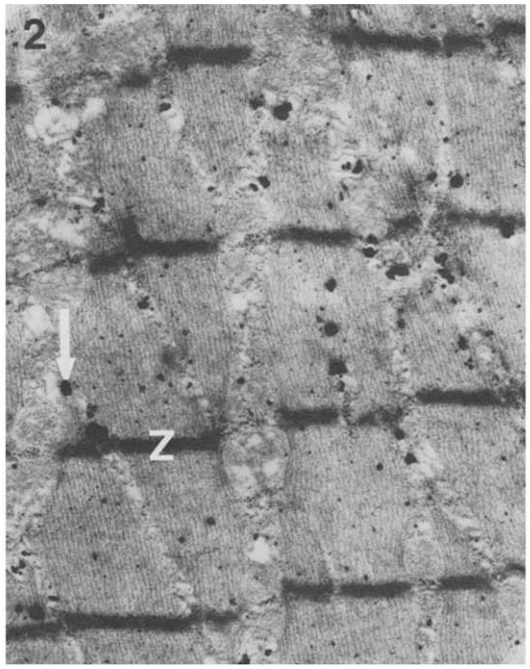

Fig.2. Contracted part of untreated muscle. Precipitates are localized in the sarcoplasmic reticulum, but outside the vesicles. × 18 900.

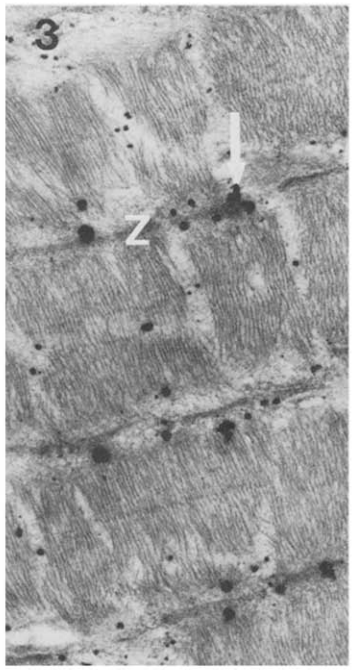

Fig.3. 2,4-D-treated muscle. Pyroantimonate deposits are found close to the Z lines. × 14 400.

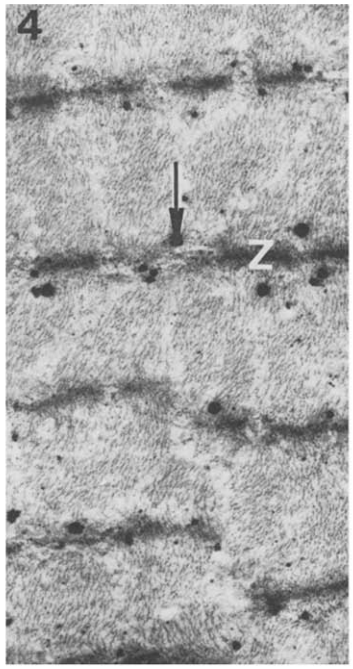

Fig.4. An advanced state of degeneration. Myofibrils are disrupted and some vacuoles are present. Dense granules are localized near the Z lines. × 14 400.

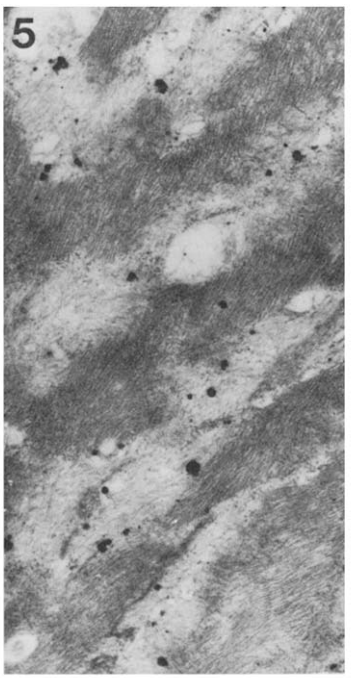

Fig.5. Part of a treated muscle with severe degeneration. × 14 400.

After a 3 week treatment with 2,4-D, the majority of fibres was contracted and changes of different severity were observed. Figure 3 demonstrates part of a white muscle in contraction. A few deposits of 250–400 Å diameter are bound to the outer surface of sarcoplasmic vesicles, but the vesicles remain empty. In contrast to the control muscle, deposits of 1000–2000 Å diameter are confined mainly to the A-I junction, near the Z line.

An advanced state of degeneration is seen in fig.4: the length of sarcomeres is variable over a short distance, no clear distinction is possible between the A and I bands, the continuity of myofibrils is disrupted and some vacuoles are present. Dense pyroantimonate granules are localized near the Z lines. Figure 5 shows part of a muscle with severe degeneration. Such degeneration was observed only in the minority of muscle fibres. Similar but less pronounced changes were also seen in the red muscle.

X-ray microanalysis in a transmission scanning electron microscope (Jem-Asid) revealed that both the smaller and the larger precipitates contained appreciable amounts of calcium.

Semiquantitative evaluation of pyroantimonate deposits was carried out by counting the smaller and larger precipitates covering 100 sarcomeres in control and 2,4-D-treated muscles. The ratio between the smaller and larger precipitates was 10:1 in the control but it changed to 10:5 after 2,4-D treatment. Parallel with the disappearance of smaller precipitates from the sarcoplasmic vesicles, an increase in the larger deposits was noted at the A-I junction, particularly in white muscle.

Kuhn and Stein [8], using ^{45}Ca, reported an inhibition in vitro by 2,4-D of calcium uptake by isolated sarcoplasmic reticulum. In the contraction–relaxation coupling troponin composed of three subunits, troponin I, C and T has an important regulatory role [9,10]. The results of our investigations, interpreted in the light of Kuhn and Stein [8], clearly suggest that 2,4-D treatment results in a change in the distribution of calcium between the triads and troponin C. When uptake in the triads is inhibited, a higher amount of calcium is attached to the troponin C, leading to a long-lasting, and finally, irreversible activation of the actin–myosin system. The degenerative changes observed in our studies after subacute 2,4-D treatment were similar to those found in acute 2,4-D poisoning of humans [11].

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