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Case Report

Modifications in the Sarcoplasmic Reticulum and Subcellular Calcium Distribution in Skeletal Muscle in a Case of Westphal's Disease (Hypokalemic Periodic Paralysis)

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Summary. In a case of hypokalemic periodic paralysis with characteristic alterations of the sarcoplasmic reticulum (SR) in the skeletal muscle, subcellular calcium re-partition, as revealed with the pyroantimonate technique, appears disturbed during paralysis. Pyroantimonate precipitates, normally concentrated in the terminal cisternae of the SR, were localized in the T tubules, whereas the terminal cisternae appeared empty. The increase (about 14%) in muscular calcium during paralysis may result from the accumulation of calcium in the extracellular compartment (T tubules). Defects in calcium uptake and storage by the SR may be involved in the pathogenesis of the periodic paralysis syndrome.

Key words: Hypokalemic periodic paralysis – Calcium – Sarcoplasmic reticulum – T tubules.

Introduction

Westphal's disease is characterized by episodic attacks of weakness with flaccid paralysis of skeletal muscles, which coincide with hypokalemia resulting from penetration of K^+ into the muscle. KCl accelerates both recovery and the outflow of intramuscular K^+. Any treatment which favours K^+ influx into the muscle (glucose, insulin, ACTH) can induce an attack of paralysis in patients with hypokalemic periodic paralysis (HPP). One pathogenic explanation is that the disturbance of K^+ permeability might lead to the accumulation of K^+

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in muscle cells, thus causing hyperpolarization and loss of excitability of the membranes (Grob et al., 1957). This hypothesis does not fit in with observations indicating depolarization of the membranes during paralysis (Hofmann and Smith, 1970). Moreover, other forms of periodic paralysis, with either hyper-or normokalemia, are known to exist. A defect in calcium regulation may be involved (Schutta and Armitage, 1969; Engel, 1970; Weller and McCardle, 1971; Au and Yeung, 1972; Ionasescu et al., 1974). Tubular aggregates reported in muscles of HPP patients (Gruner, 1966; Odor et al., 1967) are generally considered to be sarcoplasmic reticulum (SR) abnormalities and one can perhaps envisage dysfunctioning of the SR, which regulates the Ca^{2+} movements. Our observations with the pyroantimonate method on an HPP patient whose muscles displayed characteristic SR changes, suggest that the calcium storage capacity of the SR is disturbed during paralysis.

Material and Methods

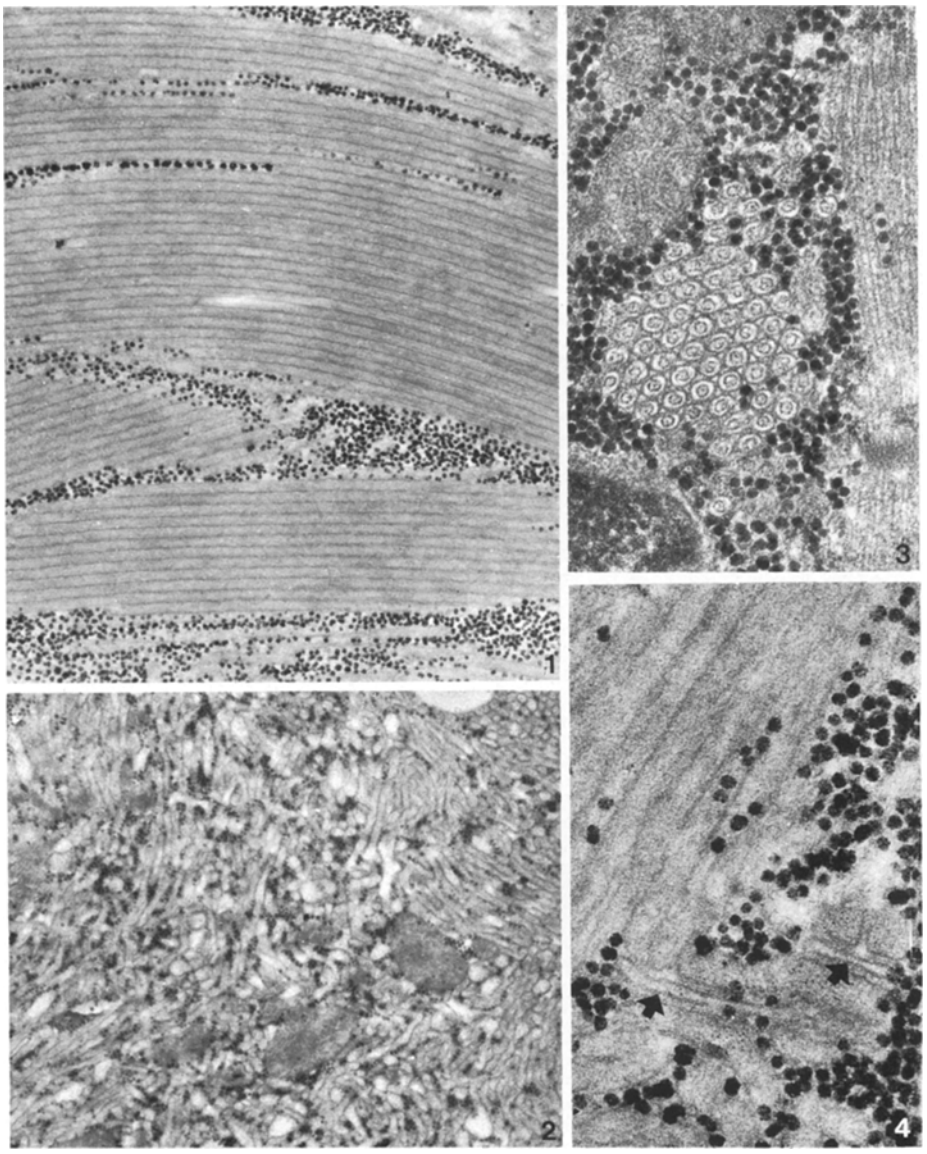
B.J., a boy of 14, has had the periodic paralysis syndrome for 2 years. His serum potassium, about 4 mEq/l under normal conditions, falls to 2 mEq/l when paralysis occurs. An attack of paralysis was experimentally provoked with the classic glucose-insulin treatment (oral glucose, 88 g.; insulin, 20 u., IM) and occurred 3 h later, coinciding with a drop in potassium (to 1.3 mEq/l). Recovery occurred 3 h later, at the same time as the serum potassium reached 3.8 mEq/l. The level of the other electrolytes did not vary, or only slightly so. Urinary elimination of Na^+, K^+, Ca^{2+}, Cl^- was approximately halved despite polyuria. Two muscle biopsies (Vastus lateralis) were taken from this patient, one before, the other $2^1/_2$ h after the onset of paralysis, for electron microscopic studies and ionic measurements. Potassium was measured with flame spectrometry (Eppendorf); calcium and magnesium by atomic absorption (Perkin Elmer 303). Fragments for electron microscopy were fixed with 5% glutaraldehyde in 0.1 phosphate buffer at pH 7.4, postfixed with 2% osmium tetroxide and embedded in a mixture of epon and aradite. For localization of calcium, samples were directly fixed in an unbuffered solution containing 1% Na pyroantimonate and 1% osmium tetroxide. Other fragments were processed with Lane's technique to localize SR ATP ase activity (Lane, 1967). The thin sections stained with uranyl acetate and lead citrate, or unstained, were examined under a Siemens Elmiskop IA.

Results

Significant penetration of potassium into the muscle occurs during paralysis (56.59 ± 2.84 to 64.12 ± 1.30 mEq/100 g dry weight). An increase in intramuscular calcium (494 to 561 ug/g dry weight) and magnesium (661 to 911 ug/g dry weight) is detected at the same time.

Light microscopic observations on semithin sections reveal disseminated PAS-positive areas, which are slightly metachromatic when stained with toluidin blue, and numerous dispersed fine lipid droplets. No difference can be seen between the two biopsies.

Under the electron microscope, these areas are seen to correspond to large accumulations of tubular structures associated with glycogen particles. At the periphery of muscular fibres, these tubules are most often tightly packed in bundles which give rise to paracrystalline formations (Fig. 1). These tubular bundles are diversely oriented, independently of the direction of the myofibrils.



Figs. 1–4. Various aspects of tubular aggregates
Fig. 1. Bundles of tightly packed tubules. **Fig. 2.** Intermingled sinuous tubules. **Fig. 3.** Transversal section showing the double outline of the tubules. **Fig. 4.** Bundle of parallel tubules, some of which terminate opposite the T tubules (*arrows*). Note relation between glycogen particles and tubular aggregates. **1** × 24,500; **2** × 15,000; **3** × 50,000; **4** × 64,000

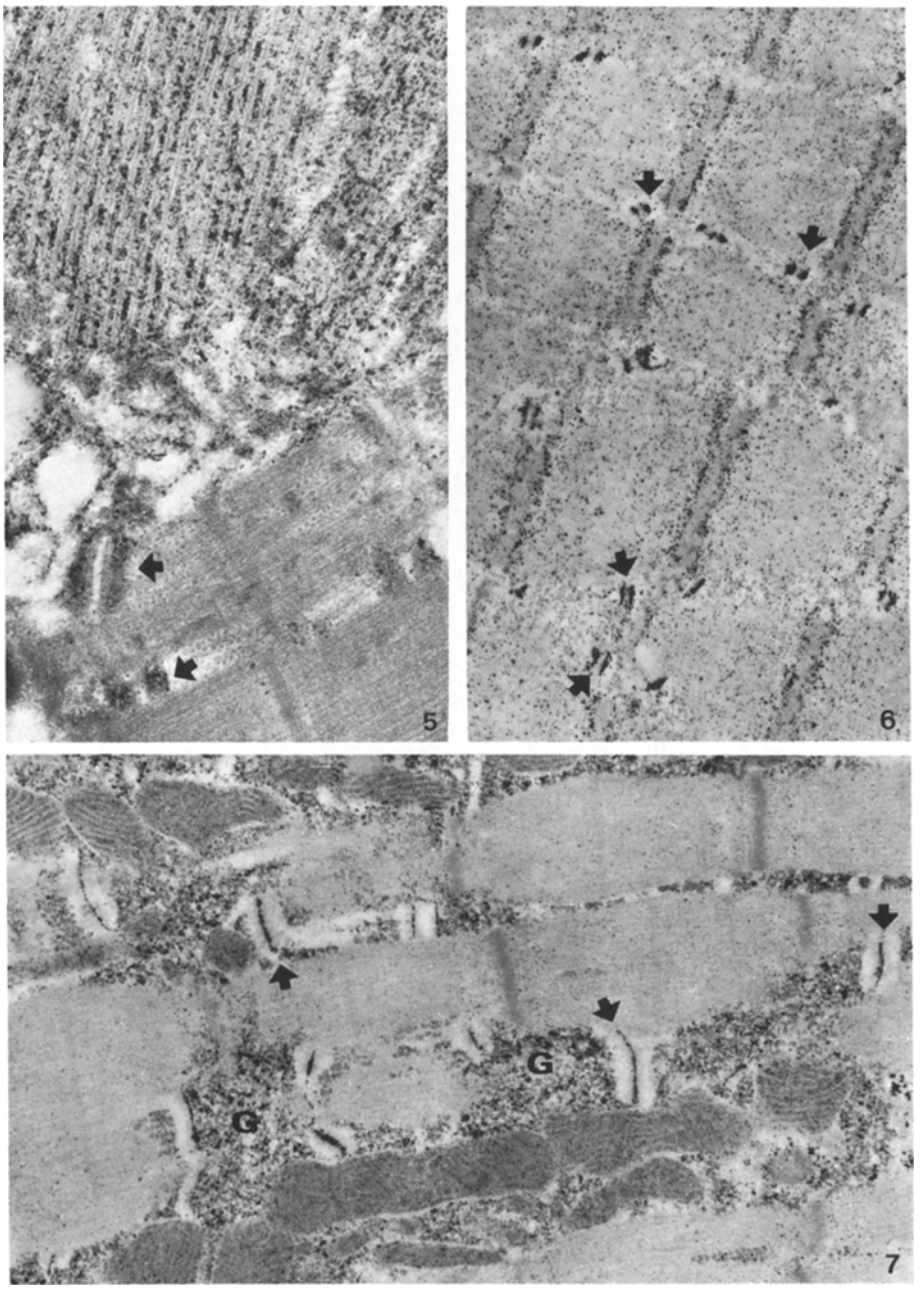


Fig. 5. Localization of ATP ase activity in the terminal cisternae of SR *(arrows)* and in the tubular aggregates. $\times 29{,}000$

Figs. 6 and 7. Localization of calcium with pyroantimonate technique. Unstained sections. **Fig. 6.** Before paralysis: PA precipitates concentrated in the terminal cisternae of SR *(arrows)* and absent from the T tubules. **Fig. 7.** During paralysis: PA precipitates present in the T tubules *(arrows)* and absent from the terminal cisternae. They are abundant, as usual, in the mitochondria but sparse in the myofibrils. Glycogen *(G)* staining is frequently observed with PA technique. **6** $\times 15{,}000$; **7** $\times 22{,}000$

Glycogen particles are concentrated around the bundles of parallel tubules. In more central areas, tubular aggregates lie more irregularly and less densely. Sinuous tubules are randomly oriented, and intermingled (Fig. 2). Numerous glycogen particles are scattered among the tubules. On transverse sections, the tubules always have a characteristic double outline (Fig. 3). Some tubules are seen to terminate in slight dilations in contact with T tubules (Fig. 4). ATP ase activity detected with Lane's technique is, as usual, localized mainly in the terminal cisternae (TC) of the SR, but also in the tubular aggregates (Fig. 5), where lead phosphate tends to precipitate in the centre of the tubules. These observations, taken as a whole, suggest that these tubular elements derive from the SR, although it has undergone considerable changes in organization and structure.

The only difference between the two biopsies is seen with the pyroantimonate (PA) technique. In the muscle taken before the attack of paralysis, PA precipitates are concentrated in the TC of SR as they are in normal muscle (Fig. 6). In the paralyzed muscle, the TC appear empty, and PA precipitates are localized in the T tubules which appear as fine dense lines (Fig. 7). This inverted localization of the PA precipitates in the triads clearly occurs in most of the muscular fibres. Variations in the fine PA precipitates related to myofibrils and mitochondria are less obvious. In the paralyzed muscle, however, they appear less concentrated in the relaxed myofibrils but not in the mitochondria. PA precipitates are not observed in the tubular aggregates.

Discussion

Investigations with the PA technique, checked with chelators and microprobe analysis, have shown that PA precipitates calcium electively in various tissues (Legato and Langer, 1969; Yarom and Chandler, 1974; Stoeckel et al., 1975). Although this technique is not absolutely specific (Klein et al., 1972; Oberc and Engel, 1977), on the basis of our experience with various tissues we nevertheless consider it suitable for calcium, provided one takes into account only precisely situated and reproducible precipitations. Coarse PA precipitates occurring haphazardly, albeit mainly in the centre of the preparations which contain various cations, should be ignored. PA clearly reveals calcium in the TC of SR where calcium linked to Ca-binding protein is known to be especially concentrated. The changes in the distribution of PA precipitates in the triads probably indicate an abnormal calcium re-partition between TC and T tubules during paralysis. The increase of about 14% in intramuscular calcium might result from accumulation of calcium in the extracellular compartment (T tubules), since the PA precipitates are rather less concentrated in the myofibrils and no more abundant in the mitochondria.

In normal muscle, calcium is mainly stored in the TC of SR, which are considered to be sites for Ca^{2+} release, which triggers off the contraction (see Fuchs, 1974; Gillis, 1977). The absence of PA precipitates from the TC during paralysis probably reveals a failure in the calcium uptake and storage capacity of the SR. This could explain the shift of calcium which accumulates in the

T tubules. On the other hand, the inverted calcium localization in the triads suggests that calcium exchanges between extra-and intra-cellular compartments occur mainly at this level and that they are disturbed during paralysis. Calcium accumulation in T tubules, like the alteration in K^+ permeability, may reflect a functional defect in the sarcolemmic membrane.

K^+ influx and paralysis cannot be clearly correlated unless the hyperpolarization theory can be proved. It is known that K^+ (and Na^+) to a certain extent control the Ca^{2+}-pumping activity of the SR (Gattnass and Demeis, 1975). The sudden penetration of K^+ might reveal a latent functional failure of the SR, as suggested by the frequent morphological abnormalities of this system (i.e., tubules with a double outline which tend to form paracrystalline aggregates).

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