The experimental induction of ultrastructural damage in cardiac muscle

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Summary. Experimentally-induced rises in intracellular calcium ($[Ca^{2+}]_i$) promote rapid myofilament degradation in amphibian and mammalian cardiac muscle strips. The relevance of these studies to the subcellular injury produced by ischaemia, the possible involvement of lysosomal enzymes and similarities with skeletal muscle are discussed.

Ischaemia and anoxia both rapidly cause cellular damage in the mammalian heart and the importance of a simple model system for studying the associated subcellular events has recently been emphasized[2]. It has been suggested that an early feature of the cellular changes is lysosome disruption[3], the consequent release of acid hydrolases precipitating intracellular damage[4]. Transient episodes of ischaemia are known to cause an acute, marked increase in $[Ca^{2+}]_i$ in myocardial cells[5]. Perfusion of the isolated rat heart for more than 2 min with Ca^{2+}-free saline causes a rapid cessation of contractile activity but readmission of Ca^{2+} initiates massive cellular damage and enzyme release (termed the calcium paradox); the damaging effects have been attributed to sudden transmembrane fluxes of Ca^{2+} (Hearse et al.[6]).

These studies show interesting parallels with the experimental induction of ultrastructural damage in amphibian[7] and mammalian[8] skeletal muscles, in which marked rises in $[Ca^{2+}]_i$, induced by the ionophore A23187 or caffeine[9], rapidly initiate myofilament dissolution. It was suggested that such a rise in $[Ca^{2+}]_i$ probably promotes lysosomal breakdown[10]. In the present studies, therefore, the action of A23187 was tested on cardiac muscle strips from both amphibians and mammals, using these as a model system for the study of the sequence of events in myocardial cellular damage.

Material and methods. Mouse (Bal/b strain, 12-20 g) cardiac muscle preparations were prepared by separating the auricle and ventricle and then slicing them into small strips in oxygenated (95% O_2:5% CO_2) Krebs solution; exposure to test agents was carried out in 8 ml of solution at 37 °C. Strips of frog *(Rana temporaria)* cardiac muscle were similarly treated in Ringers solution at 22 °C. Isolated frog hearts were prepared for perfusion and recording by conventional techniques; the saline contained (mM): NaCl 110, KCl 1.88, $CaCl_2$ 1.8, $NaHCO_3$ 1.43, Na_2HPO_4 0.07, buffered to pH 7.4 using NaH_2PO_4. Fixation and treatment for electron microscopy followed the procedure given by Duncan and Smith[9].

Results and discussion. Exposure of both amphibian and mammalian cardiac muscle strips to A23187 over a range of concentrations and exposure times usually produced little or no sign of ultrastructural damage, in marked contrast with the action of the ionophore on skeletal muscle[7,8]. However, if, during a 5-10-min exposure to A23187 (2.5 μg ml^{-1}), the cardiac muscle strips were partially depolarized by raising $[K^+]_o$ to 20 mM, or were made to contract by field stimulation (10 stimuli sec^{-1} for 10 min),

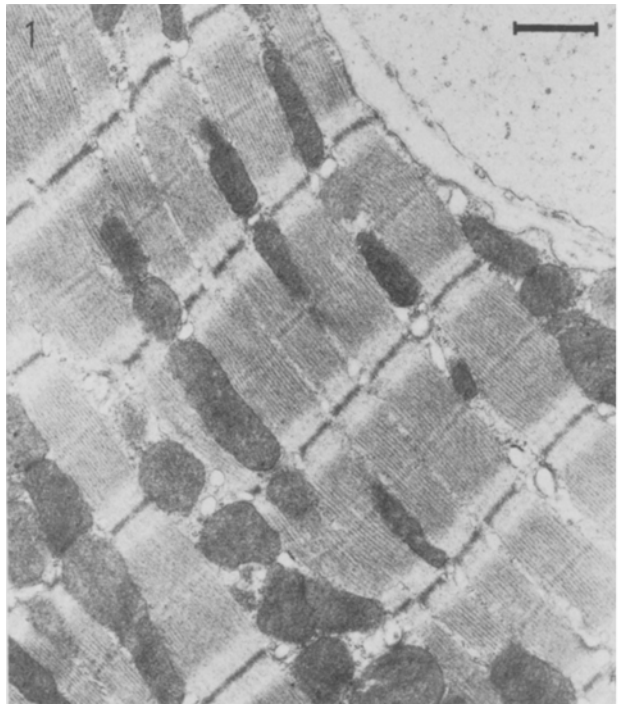

Fig. 1. Electron micrograph of a control mouse ventricle strip exposed in saline for 20 min at 37 °C. Bar = 1 μm.

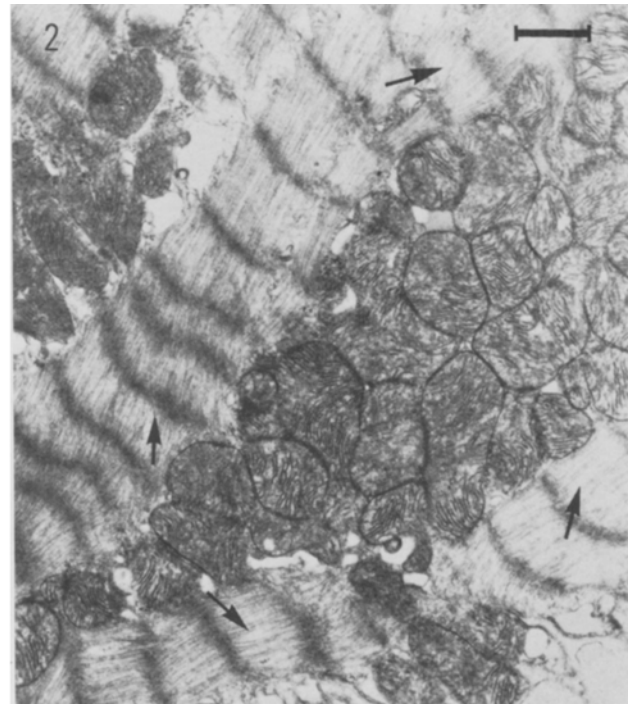

Fig. 2. Electron micrograph of a mouse ventricle strip exposed for 15 min in saline containing 2.5 μg ml^{-1} A23187 at 37 °C followed by partial depolarization in saline containing 20 mM K^+ and 2.5 μg ml^{-1} A23187 at 37 °C for 10 min. Arrows show blurring of Z-lines, myofilament degradation and, in particular, loss of the myosin filaments. Bar = 1 μm.

extensive myofilament degradation was always found in both mammalian and amphibian preparations which closely resembled that described in skeletal muscle[7–9]. Typical damage included intensely contracted filaments with blurring of the Z-lines (see figures 1 and 2); in other areas, extensive myofilament dissolution was seen, with the myosin filaments being particularly affected. Finally, areas could be found with complete degradation of the myofilament apparatus. Control preparations similarly treated but lacking ionophore were normal.

We conclude that marked rises in $[Ca^{2+}]_i$ in cardiac muscle are able to initiate myofilament degradation, similar to that reported in skeletal muscle. However, A23187 rarely causes damage in cardiac muscle except when its action is augmented by partial depolarization or stimulation. The ionophore is effective with skeletal muscle in the absence of extracellular Ca^{2+} and is believed to act primarily by releasing Ca^{2+} from the sarcoplasmic reticulum (SR). We suggest that this difference between skeletal and cardiac muscle may lie in the relative importance of the different systems controlling $[Ca^{2+}]_i$. The bulk of the intracellular Ca^{2+} is stored in the SR in skeletal muscle which is therefore particularly sensitive to A23187; in cardiac muscle the mitochondria contain (Patriarca and Carafoli[11]) the Ca^{2+} required for contraction enters from outside the cell at excitation. We conclude that release of Ca^{2+} from the SR by A23187 does not usually raise $[Ca^{2+}]_i$ to sufficiently high levels in cardiac muscle to cause rapid myofilament degradation, unless accompanied by an influx of extracellular Ca^{2+} induced by the depolarization of the plasma membrane.

Exposure of amphibian or mammalian skeletal muscle strips to either 2,4-dinitrophenol (DNP) (10^{-4} M, 25 min) or to ruthenium red (30 µM, 40 min) also rapidly causes typical myofilament degradation with the same characteristics as those described above. So far, we have not found any evidence of reversibility of this rapid degradative effect in our in vitro systems.

Equally, perfusion of the isolated frog heart with saline containing 10^{-4} M DNP caused cessation of contractile activity in 3–5 min and electron microscopy of the muscle at this stage again revealed myofilament degradation. Contractile activity continued on perfusion with 10^{-5} M DNP, but examination again revealed extensive damage. DNP is a mitochondrial uncoupling agent, and ruthenium red inhibits intracellular Ca^{2+}-uptake, and we conclude that ultrastructural damage can be rapidly produced in cardiac muscle when the functional integrity of the mitochondria is seriously impaired and that the consequent release of Ca^{2+} from this major intracellular store[11] is a potent means of triggering damage.

From these studies, we suggest that 1. Cardiac and skeletal muscles are similar in that myofilament damage can be rapidly induced by a variety of treatments in both. 2. The common feature in these experiments appears to be a marked rise in $[Ca^{2+}]_i$ in the myoplasm. 3. The 2 tissues differ in the relative importance of the different intracellular systems controlling $[Ca^{2+}]_i$. The SR constitutes the main store of Ca^{2+} in skeletal muscle; in cardiac muscle the mitochondria (as is shown in ischaemia or DNP treatment) and the plasma membrane (as in the calcium paradox) are of particular importance. The sensitivity of mitochondrial Ca^{2+} accumulation to ischaemia in cardiac muscle is well documented. 4. A marked rise in $[Ca^{2+}]_i$ probably promotes lysosomal breakdown in cardiac and skeletal muscle, the enzymes so released being responsible for this form of rapid myofilament degradation[10]. Methyl prednisolone is a known lysosomal stabilizing agent[12] which has been shown in some studies to have a protective effect in the damage induced by ischaemia in cardiac muscle[3]. 5. These findings could be of importance for our understanding of cellular injury in cardiac muscle following ischaemia.

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Photoreceptor properties of an ectopic eye in the fleshfly, *Sarcophaga bullata*[1]

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Summary. Ectopic eyes were produced on the fleshfly, *Sarcophaga bullata* by transplantation of imaginal eye discs. Electrophysiological and histological observations of these supernumerary eyes indicate the absence of synaptic connections between retinular cells and higher order neurons.

The compound eye of insects consists of several thousand ommatidia, and each ommatidium is composed of photoreceptor cells called retinular cells. Determining the properties of these sensory cells by extracellular recording has been complicated by potentials originating from synaptic connections with second order neurons. Studies with surgically isolated ommatidia[3] and with eyes cultured in vivo (and hence lacking synapses)[4] have indicated that photoreceptor cell depolarizations contribute a monophasic negative response to the electroretinogram (ERG, sometimes called the retinal action potential). In this study we have investigated the retinular cells by using an ectopic eye in the fleshfly, *Sarcophaga bullata.* These eyes closely resemble the normal eyes in their external appearance.