Histoenzymological study of myocardial ATPase activity in experimental infarction in the rat


(Received January 23, 1981)

Summary

Histoenzymological techniques were used to examine ATPase activity in rat heart muscle fibres after experimental infarction. 24 hours after coronary ligation, ATPase activity in all ventricular section fibres was high, homogeneous at pH 9.4, and inhibited by acid preincubation, and the same was observed for control heart sections. 48 hours after ligation, necrotic ventricular fibres appeared, leaving only a thin layer of fibres which had apparently preserved their myofibrillar ATPase. These results indicate that, unlike mitochondrial enzyme activity, myofibrillar ATPase activity is relatively resistant to ischaemia.

Key words: rat, infarction, ATPase, histoenzymology

Introduction

Histoenzymological methods have extended our knowledge of the morphological and biochemical changes occurring during experimental myocardial infarction. For instance, a defect in mitochondrial oxidative enzyme activity was demonstrated histoenzymologically a few hours after coronary ligation, whereas routine histological techniques only showed moderate lesions (1, 5, 12). Little information has so far been published about the enzymatic activity of contractile apparatus, with the use of histoenzymological methods. However, during the irreversible step of myocardial infarction, ultrastructural studies did reveal a profound change in myofibrillar material (6, 18). Biochemical studies reported a decrease in the myofibrillar (2) and myosin ATPase after myocardial infarction (13, 19), but a structural analysis made 48 hrs after coronary ligation in the rat reported that the myosin appeared unchanged by electrophoresis (2). The reasons for these discrepancies are not known. Unlike histoenzymological techniques, biochemical methods do not per-
mit to specify the exact location of the infarcted area of the heart, neither do they make it possible to determine whether or not the lesions are homogeneous at a given moment or during their evolution (11).

Histoenzymological techniques were chosen for this work, for the following purposes:
- to examine regional changes in ATPase activity in rat myocardium after experimental coronary ligation, and
- to determine whether these changes were homogeneous in the different regions of the ischaemic myocardium and to compare the results with biochemical data (2, 13, 19).

With this in view, ATPase activity was studied at pH 9.4 by a well-known histoenzymological method (16), and after acid preincubation (3).

Material and methods

I Experimental procedure

Studies were performed on male Wistar rats weighing 200 to 250 g. Myocardial infarction was obtained by the method of Johns and Olson (1954) (15), modified by Deloche and coll. (9).

Briefly, each rat was anaesthetized with ether. Left thoracotomy was performed; the heart was exteriorized by gentle pressure on the right side of the thorax. The left coronary artery was ligated 2-3 mm from its origin. The heart was then returned to the thorax, which was immediately closed. Operative mortality was around 30%.

24 or 48 hrs after surgery, rats were killed by intravenous sodium pentobarbital injection and hearts were quickly removed. Six rats were studied for each experimental period. Seven normal animals were used as controls. No sham-operated rats were studied since Fischbein et al. (12) showed that there was no morphological difference between sham-operated and normal animals.

II Morphological techniques

Immediately after excision, a 2 mm thick cross-sectional heart slice was cut 3 to 4 mm from the apex and rapidly frozen in isopentane prechilled with liquid nitrogen. 8 µm thick cryostat serial sections (Microtome Damon I.E.C., Weedham, USA) were made and mounted. They were dried for 20-30 min at room temperature and stained by the histoenzymological and histological techniques described below.

1 Demonstration of ATPase activity

a) Demonstration of ATPase activity at pH 9.4 was performed according to Padykula and Herman (16). Briefly, sections were incubated for 15 min. at 37 °C in the following medium: 5 mM ATP (Sigma Chemical Company, St.-Louis, USA), 18 mM CaCl₂ and 10 mM sodium barbital (Prolabo, Paris, France). Preliminary experiments had shown that an incubation time of 15 min gave the most satisfactory results. Sections were washed in a solution of 1% CaCl₂ (3 changes, 1 min. each), drained, placed for 3 min in a solution of 2% CoCl₂ (Merck, Darmstadt), washed four times for 1 min in distilled water, immersed in a solution of 1% ammonium sulfide for 2 min, again washed with tap water, quickly dehydrated with alcohol and toluene and mounted in permount.

b) Demonstration of ATPase activity after preincubation at pH 4.35 was performed according to Brooke and Kaiser (3). Some sections were preincubated in sodium acetate buffer at pH 4.35 for 10 min at room temperature, washed in distilled water and incubated for another 15 min at 37 °C in the medium used to reveal ATPase activity at pH 9.4. Subsequent procedure was similar to a).