Z. Rechtsmed. 83, 225—232 (1979)

Zeitschrift für
Rechtsmedizin

Investigation of the Short-Time Autolysis of Rat Hearts by Means of SDS

Polyacrylamide Gel Electrophoresis and Electron Microscopy

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Summary. The short-time autolysis of hearts was regarded as a model of ischaemic heart failure. Therefore, isolated rat hearts were subjected to 30—120 min autolysis in a Locke solution at 37°C. Electron microscopic examinations and myofibrillar preparations were made from the autolysed heart ventricles. The myofibrillar proteins were resolved by SDS-polyacrylamide gel electrophoresis. After 30 min autolysis the amount of a protein of 192,000 daltons greatly increased. At the same time on the electron micrographs the focal destruction of filaments adhering to intercalary discs could be observed as well as a focal filament destruction on the A filament area and the mitochondrial structure altered too. After 60 min autolysis another protein of 36,400 daltons appeared. On the electron micrographs the focal desintegration of Z membranes and the focal destruction of I filaments can be observed. After 120 min autolysis further proteolytic products could not be detected by gel electrophoresis but on the electron micrographs the destruction of Z membranes and I filaments became more pronounced.

Key words: Autolysis, rat heart – Ischaemia, short-time autolysis as a model

Zusammenfassung. Die Kurzzeitautolyse des Myocard wird als Beispiel für einen Hypoxieschaden betrachtet. Isolierte Rattenherzen wurden einer Autolyse von 30, 60 und 120 min in Lockescher Lösung bei 37°C unterworfen und elektronenmikroskopische Untersuchungen sowie eine Präparation der Myofibrillen durchgeführt. Die Proteine der Myofibrillen wurden durch SDS-Polyacrylamid-Gel-Elektrophorese aufgetrennt. Nach 30 min Autolyse fand sich eine Zunahme eines Proteins von 192 000 Dalton. Zur gleichen Zeit konnte elektronenmikroskopisch sowohl eine fokale Zerstörung der Filamente, die zu den Zwischenscheiben gehören, als auch die fokale Zerstörung der Filamente im Bereich der A-Filamente beobachtet werden. Auch änderte sich die Mitochondrienstruktur. Nach 60 min Autolyse erschien ein weiteres

0044-3433/79/0083/0225/$ 1.60

Protein von 36400 Dalton. Elektronenmikroskopisch konnte eine fokale Desintegration der Z-Membranen und eine fokale Zerstörung der I-Filamente beobachtet werden. Nach 120 min Autolyse fanden sich elektrophoretisch keine weiteren proteolytischen Produkte, aber elektronenmikroskopisch zeigte sich eine Zerstörung der Z-Membranen und I-Filamente.

Schlüsselwörter: Autolyse, Rattenmyocard – Hypoxie, Kurzzeitautolyse als Beispiel

The pathological alterations of muscle and myocardium induce changes in the field of contractile proteins, too. Among the myocardiac processes the ischaemic damages were investigated most abundantly because of their clinical significance. Many authors [1, 2, 3, 13, 14, 15, 26] pointed to the parallelism of the first steps of autolysis and that of ischaemic injuries, so conclusions can be drawn from the first steps of autolysis to the pathological alterations of the ischaemic cardiac muscle.

In our previous paper [25] the myofibril proteolytic products of autolysing skeletal muscle were investigated. In our present work we are trying to compare the alterations of the proteins of myocardiac myofibrils with the changes of the electron microscopic observations during the first 120 min of autolysis.

Material and Methods

Male rats (180—200 g body wt) were killed by stunning and the whole hearts were quickly removed and placed in a Locke solution (0.09% NaCl, 0.042% KCl, 0.024% Ca_2Cl, 0.10% glucose, 0.020% $NaHCO_3$) which was kept at 37°C. The rats were not anaesthetised prior to killing. The hearts were autolysed for different times. Four different groups were used each consisting of 5 rats. The first group served as control, zero time autolysis. In the second group the hearts were autolysed for 30 min while in the third and fourth groups the hearts were autolysed for 60 and 120 min, respectively.

In the first group immediately after the killing of the rats and in the other groups at the end of the autolysis the left heart ventricles were cut out and worked up for myofibril preparations and for electron microscopic examinations.

For myofibril preparations the fresh and the autolysed heart ventricles were prepared according to Perry [18] with some minor modifications [24]. The protein content of the myofibrillar samples was determined [6] and following the solubilisation of the samples [19] they were subjected to SDS-polyacrylamide gel electrophoresis [27]. Each myofibrillar sample was runed on two different gel columns one containing 4% and the other 10% acrylamide gel. To resolve myofibrillar proteins with higher molecular weights than that of the actin the former gels are more suited and for proteins with lower molecular weights than that of the latter gels are better.

The identification of the myofibrillar proteins was accomplished according to their molecular weights by the aid of marker proteins [27].

The samples for electron microscopy were taken out from the middle zone of the left ventricle muscle and immediately after the removal they were fixed in glutaraldehyde and osmium tetroxyde, and embedded in Durcupan. The ultrathin sections were contrasted with uranyl acetate and lead citrate. Electron micrographs were taken on a Jeol 100 B electron microscope at 80 kV.

Results

The protein components of the myofibrils prepared from rat heart ventricles and resolved on gel columns containing 4% acrylamide can be seen in Fig. 1. The myofibrillar proteins of the fresh heart ventricles are in turn from above to downwards (the molecular weights given in daltons are in parenthesis): the myosin heavy chain (206,000), the following two proteins probably the Mα-line and the Mβ-line proteins (192,000 and 175,000), an unknown protein (156,000), the C-protein (144,000), a protein which probably remained from the sarcoplasmic reticulum [18] and might be the Ca^{++} activated ATPase (109,000), the α—actinin (98,000), two unknown proteins (75,000 and 52,000) and the actin (44,000).

The muscle samples autolysed for 30, 60, and 120 min contained those myofibrillar protein components which were present in the fresh muscle, but in each autolysed sample the quantity of the protein component of 192,000 daltons were greatly increased.

Considering the cardiac myofibrillar proteins one must take into account that in case of mammals the molecular weights of troponins, tropomyosin, and myosin light chains differ from the values obtained from the skeletal muscles of the same species. Data of some authors concerning with molecular weight of the above mentioned cardiac myofibrillar proteins and our data are summed up in Table 1.

The protein components of the myofibrils prepared from rat heart ventricles and resolved by gel columns containing 10% acrylamide can be seen in Fig. 2. The myofibrillar proteins of the fresh heart ventricles are from above to downwards: actin (44,000), troponin-T (41,000), tropomyosin (34,500), troponin-I (30,500), unknown protein (27,800), myosin light chain-1 (26,100), troponin-C (21,400), myosin light chain-2 (17,200), two unknown proteins (14,000 and 12,400) can probably be the product of an endogenous proteolysis during the preparation procedure.

All the myofibrillar proteins of the fresh muscles are present in the autolysed samples. Among the myofibrillar proteins of the muscles autolysed for 60 and 120 min there is a new proteolytic product with a molecular weight of 36,400.

To summarize the results of the gel electrophoresis one can establish that by autolysing the rat heart at 37°C in a Locke solution, already after 30 min the amount of the protein of 192,000 daltons greatly increased, after 60 min a new proteolytic product of 36,400 daltons appeared. After 120 min the myofibrillar proteins were the same as after 60 min, new products were not present.

On the electron micrographs the most striking feature was the focal destruction of filaments adhering to intercalary discs after 30 min autolysis (Figs. 3 and 4). On the area of A filaments near the border of A and I filaments patchy filament lesions and structure destructions can be observed. These alterations are irregular in extending only over a part of sarcomers. Simultaneously mitochondrial swelling and the straightening of the mitochondrial cristae as well as focal amorph substances in the matrix can be observed. After 60 min autolysis patchy desintegration of Z membrane occurs which is manifested in its irregularity and even sometimes in its disappearance. Parallel to this phenomenon, the focal destruction of thin filaments can be observed sometimes only in some parts

Table 1. The moleculur weight of some cardiac myofibrillar protein components determined by SDS-polyacrylamide gel electrophoresis

	Tropomyosin	Troponin-T	Troponin-I	Troponin-C	Myosin light chain-1	Myosin light chain-2
Rabbit	32,000 [7]	40,000 [12] 40,000 [5]	29,000 [23] 30,000 [12] 28,000 [5]	20,000 [12]	27,000 [11] 26,800 [21]	19,000 [11] 17,800 [21]
Cattle		40,000 [12] 40,000 [5]	30,000 [12] 28,000 [5]			
Dog					28,000 [26] 28,000 [17]	18,500 [28] 22,000 [17]
Rat	32,000 [16] 36,000 [22]	38,000 [22]	31,000 [22]		27,000 [8] 24,000 [22]	18,000 [8] 18,000 [22]
Rat (own results)	34,500 ± 470	41,000 ± 940	30,500 ± 570	21,400 ± 360	26,100 ± 150	18,200 ± 250

Explanation: The Figures on the right side of the molecular weights are the reference numbers. Our own results are the arithmetic mean of ten different determinations ± standard deviation

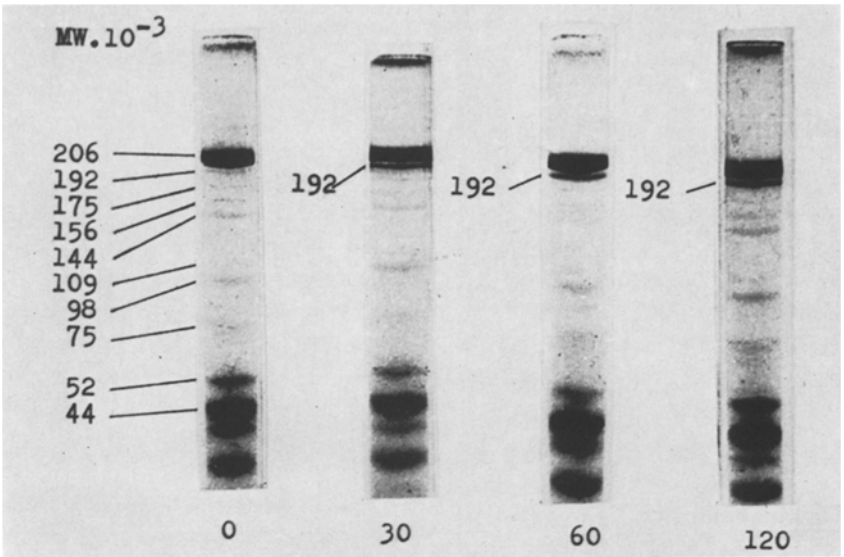

Fig. 1. From left to right the myofibrillar proteins of the fresh and the 30, 60, and 120 min long autolysed rat heart ventricles. Resolved by electrophoresis on 4% acrylamide and 0.1% SDS containing gel columns. The Figures on the left side of the gels are the molecular weights $\times 10^{-3}$ of the myofibrillar proteins

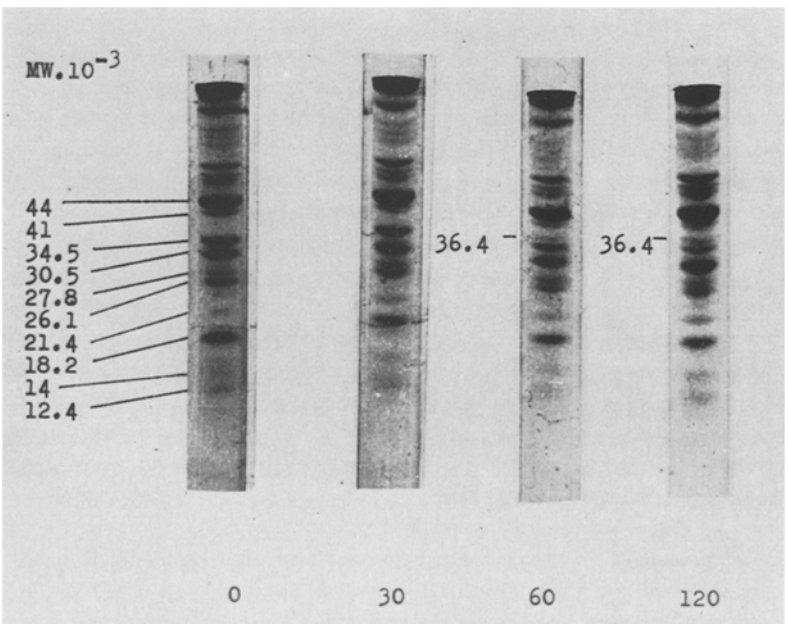

Fig. 2. From left to right the myofibrillar proteins of the fresh and the 30, 60, and 120 min long autolysed rat heart ventricles. Resolved by electrophoresis on 10% acrylamide and 0.1% SDS containing gel columns. The Figures on the left side of the gels are the molecular weights $\times 10^{-3}$ of the myofibrillar proteins

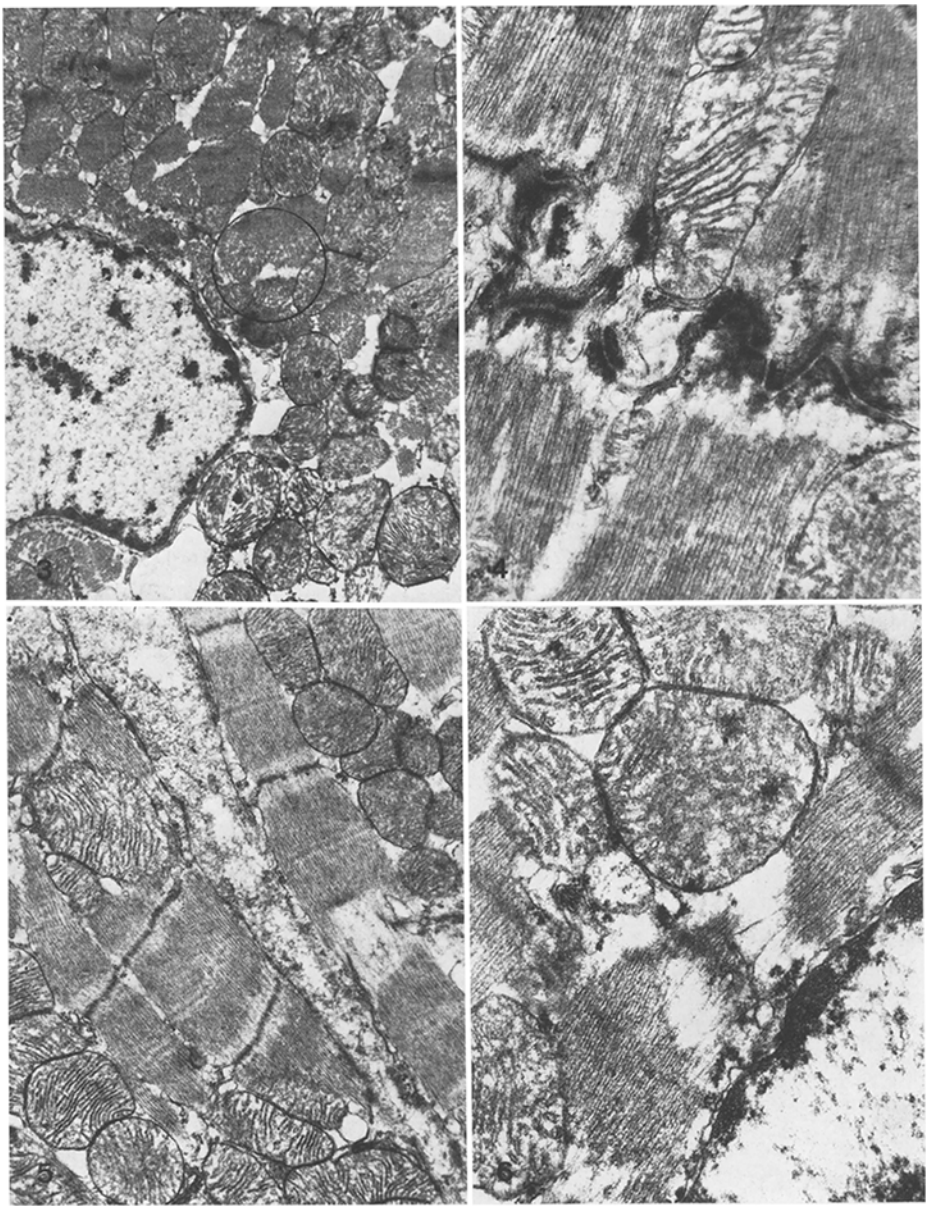

Fig. 3. 30 min autolysis, cross section. Focal destruction on thick filament region, × 8,000

Fig. 4. 30 min autolysis, longitudinal section. Focal filament destruction on the region of filaments adhering to intercalary discs. The mitochondriae are swollen, the cristae are straightened, ×32,375

Fig. 5. 60 min autolysis. Focal destruction on the areas of Z membranes and I filaments, × 16,000

Fig. 6. 120 min autolysis. Filament destruction extended to the A, partly to the I filament area. Amorph substances in the mitochondria, ×37,200

of the sarcomers (Fig. 5). After 120 min autolysis these alterations can already be observed nearly everywhere. In some places the total destruction of Z membranes and I filaments can be seen (Fig. 6).

Discussion

On the electron micrographs after 30 min autolysis the mitochondrial alterations and the destruction of filaments adhering to intercalary discs as well as the lesion of A filaments can be well observed. At the same time all the autolysed samples contained a considerable amount of a protein of 192,000 daltons just under the myosin heavy chain band. Similar molecular weight to this has the Mα-line of the rabbit skeletal muscle [20]. It is conceivable that from the autolysed heart muscle the Mα-line protein is easier to extract than from the fresh one. But it is conceivable, too, that this protein in the hydrolytic product of the myosin heavy chain and this product was resolved on the same place as otherwise the Mα-line protein took place. The focal destruction of myosin filaments on the electron micrographs would rather tempt to the latter assumption.

The autolysis of an isolated heart in Locke solution is different from the autolysis of a heart or of a skeletal muscle which was remained in the carcass. If the skeletal rabbit muscle was remaining for 24 h at 25°C in the rabbit carcass, the 192,000 daltons protein should not be detected [25]. In a complementary experiment we left the rat hearts in the carcasses for 120 min, in this case the band of the 192,000 daltons myofibrillar protein was as faint as in the fresh samples.

The different way of organs proteolysis inside and outside the carcass can be explained by the different pH conditions. Drabikowsky et al. [9] proved that the course of endogenous proteolysis was to a great extent function of pH.

After 60 min autolysis the destruction of the Z membrans and the I filament can be observed on the electron micrographs. At this time a new proteolytic product appeared with a molecular weight of 36,400. This new protein was probably the proteolytic product of troponin-T [10]. Following 120 min of autolysis, the destruction of Z membranes and I filaments became more pronounced but among the myofibrillar proteins no further proteolytic product could be detected by gel electrophoresis.

Acknowledgements. We wish to give our thanks to Prof. E. Somogyi, Chairman of the Institute of Forensic Medicine, Semmelweis School of Medicine, Budapest, Hungary, for making his electron microscopic laboratory available to us.

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Received October 25, 1978