ULTRASTRUCTURAL CHARACTERISTICS OF DIVISION OF ADULT RAT CARDIOMYOCYTE NUCLEI

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UDC 616.127-018.1-053.8-092.9-076.4

KEY WORDS: rat cardiomyocytes, nuclei, varieties of division.

The possibility of hyperplasia of the cardiomyocytes (CMC) in adult mammals during regeneration and hypertrophy is not yet considered to be firmly established [11]. Definite differences have been described in the behavior of CMC in animals of different species and in man under these conditions [10]. As regards the ventricular and atrial CMC of rats, the data obtained by thymidine autoradiography, cytospectrophotometry of nuclear DNA, and histological study of sections and of isolated cells, are quite contradictory [1-3, 5-7, 10, 12-14]. At the ultrastructural level the mitotic form of division of the nuclei of rat atrial CMC during left ventricular myocardial infarction has been analyzed [10]. Other types of division, mentioned in the literature [2, 3] and arousing basic objections [4, 10, 15], have not been investigated for this particular purpose electron microscopically. It was therefore considered worthwhile to undertake such an investigation.

EXPERIMENTAL METHOD

Nuclei of CMC were studied in 29 noninbred albino rats weighing 150-220 g. Myocardial infarction of the left ventricle was produced experimentally in 25 rats by ligation of the decending branch of the left coronary artery (group 1), and four rats were trained to swim carrying a load (5-45 min for 22 days; group 2). The animals were killed by decapitation under ether anesthesia. The zone of the left ventricle and auricles of the atria in the rats of group 1, outside the zone of infarcation, was investigated between 10 min and 10 days after the beginning of the experiment, and the myocardium of both ventricles from the animals of group 2 was studied 24 and 38 days after the end of the loading test. Pieces of tissue were fixed in glutaraldehyde and OsO_4, processed in the standard manner, and embedded in a mixture of epoxide resins. Ultrathin sections, after double staining, were studied in the "Tesla BS-500" electron microscope.

EXPERIMENTAL RESULTS

Mitotic figures of nuclear division were not found in the atrial and ventricular CMC of rats of either group. Other changes in the nuclei, suggesting their division, were observed at times of the experiments in both atrial and ventricular CMC. Several different versions of these changes can be distinguished.

The first version is the formation of a deep constriction band around the nucleus with rounded edges. The structure of the nuclear membrane in the zone of constriction is the same as in other parts of the nucleus. Sometimes condensed chromatin is folded in the middle part of the constriction ring, and apparently points to a boundary between the two parts of the nucleus, each often having its own nucleolus. In some nuclei with a constriction ring one of the parts contains an intranuclear inclusion, surrounded by a membrane (Fig. 1a). The second version is one of slit-like invagination of the nuclear surface: this may be unilateral, reaching as far as the opposite edge of the nucleus, or bilateral, meeting in the thickness of the nucleus. In both cases parts of the nucleus frequently remain connected to one another only by the narrow isthmus. The slit-like invagination more frequently has a transverse orientation (Fig. 1b), less frequently it lies parallel to the long axis of the nucleus. Condensed chromatin sometimes accumulates along the edges

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(Presented by Academician of the Academy of Medical Sciences of the USSR, D. S. Sarkisov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 106, pp. 369-371, September, 1988. Original article submitted January 20, 1988.

0007-4888/88/0009-1351$12.50

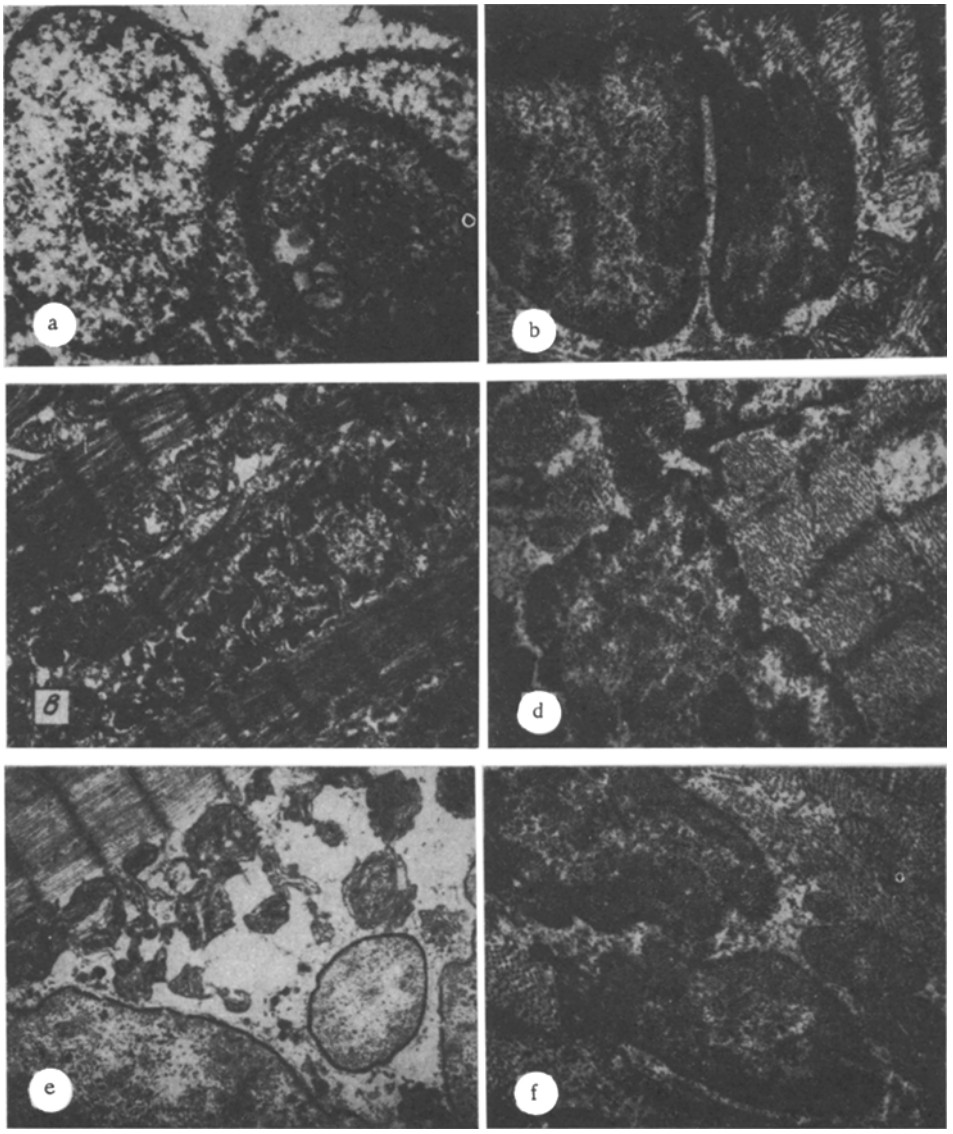

Fig. 1. Changes in nuclei of atrial (a, c) and ventricular (b, d-f) CMC. a) Deep constriction ring with rounded edges, intranuclear inclusion in right part. 14,000 ×; b) slit-like invagination, at right angles to long axis of nucleus. 10,000 ×; c) separation of nucleus into fragments. 10,000 ×; d) long isthmus between nuclei. 14,000 ×; e) micronucleus. 8000 ×; f) closely apposed nuclei with complementary surface. 14,000 ×.

of the invagination or in the zone of the isthumus. The patterns described are often found in long nuclei and lead to the formation of large or several small fragments (Fig. 1c). The third version consists of nuclei connected together by a long isthmus. The surface of the isthmus is covered with a nuclear membrane (Fig. 1d). The fourth version has a micronucleus between two nuclei of the usual size (Fig. 1e). The fine structure of all these nuclei does not differ significantly. Finally, the fifth version consists of nuclei in close apposition, often with complementary outlines of their contiguous surface (Fig. 1f).

The absence of a pattern of mitotic division of the nuclei in the ventricular CMC does not contradict the results of the majority of investigations conducted at the light-optical level, which demonstrated a very low mitotic index under similar conditions [2, 10]. Meanwhile many workers have observed mitotic division of nuclei comparatively often in atrial CMC [3, 10, 13], although there are exceptions here also [5]. The ability of atrial CMC to undergo mitotic division may perhaps be manifested not in all rats or not constantly.

The changes in the nuclei described above can be interpreted in several ways. For example, the first version can be regarded equally as a manifestation of direct division and, conversely, a sign of fusion of interphase nuclei. Such a phenomenon has been admitted in

CMC [10]. However, intranuclear inclusions found in some nuclei with constriction rings, and which are a sign of maximal destruction [9], suggest separation of the altered part of the nucleus from the intact part rather than fusion of a destructive nucleus with a normal nucleus. Consequently, the formation of a constriction ring with rounded edges may in principle be evidence of separation of the nucleus into parts, whose fate will depend on a number of factors, including on the presence of a full set of chromosomes in each part. The second version, in our view, is a reflection of the process of direct division of interphase nuclei. In both this and the first version, condensed chromatin has some part to play in division of the nuclei, for accumulation of chromatin sometimes precedes division itself.

The patterns of the final stages of direct division may be long isthmi between neighboring nuclei in close apposition to one another with complementary adjacent surfaces. How viable the nuclear fragments formed under these circumstances may be will depend, just as in the first version, on their possession of a complete set of genetic information. In view of evidence of the orderly distribution of chromosomes close to the membrane of interphase nuclei [8] and of the presence of polyploid nuclei in rat CMC [10], such a possibility is not unlikely. Long isthmi between nuclei may have a dual origin and maybe regarded not only as the final stage of direct division of nuclei, but also as one of the manifestations of pathology of mitosis. This last hypothesis has supporters [4] and it has been extended to an idea of the mechanism of formation of micronuclei [15]. But since mitoses of CMC nuclei have not been found at the times of the experiments studied, it must be accepted that the disturbances affected those mitoses which took place earlier, and in the case of ventricular CMC, probably within the first weeks of life of the animals, when mitoses were not completely blocked [10]. However, this long persistence of nuclear anomalies is doubtful. The closely apposed nuclei can also be regarded not only as the final stage of direct division, but also as the result of mitotic division, taking place under conditions of overcrowding [10]. However, the doubts expressed above, connected with the absence of mitoses in CMC nuclei in the rats studied, apply also to this situation.

The results of the electron-microscopic study thus indicate that, under the experimental conditions studied, changes are found in atrial and ventricular CMC of adult rats in whose interpretation the possibility of direct division of interphase nuclei cannot be ruled out. The fate of the nuclear fragments thus formed is not sufficiently clear and requires further investigation.

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