# ULTRASTRUCTURAL CHARACTERISTICS OF SPECIFIC GRANULES OF RAT ATRIAL CARDIOMYOCYTES

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UDC 611.125.018.63:612.172.018

KEY WORDS: specific granules; cardiomyocytes; atrium.

The presence of specific granules (SG), morphologically similar to secretory inclusions of endocrine cells, in mammalian atrial cardiomyocytes suggests that SG are linked with the endocrine function of the atria [5]. Special musculosecretory cardiomyocytes, characterized by the poor development of their myofibrillary apparatus but by a large number of SG, have even been described [1]. Interest in the study of atrial cardiomyocytes increased after a hitherto unknown natriuretic hormone, with a vasodilator action, was isolated from the atrium [7, 9]. Investigation of the nature of this hormone stimulated the search for the structural elements responsible for its synthesis or accumulation. A definite role in this respect has been finally settled. Some investigators claim that SG are related to catecholamine metabolism [4].

The aim of the present investigation was to determine the composition of SG, and in particular, whether they contain catecholamines and proteins, both under normal conditions and during exposure to various factors.

## METHODS

SG was studied in cardiomyocytes from the auricle of the right atrium of 42 noninbred albino rats weighing 180-200 g. The animals were divided into five groups: 1) control (n = 12), 2) receiving adrenalin (n = 8), 3) receiving rausedil (n = 8), 4) hemodynamic loading of the heart (n = 6), and 5) receiving rausedil + hemodynamic loading of the heart (n = 8).

Adrenalin, in a dose of 1 mg/kg, was injected intraperitoneally. The rats were killed 3 h after a single injection or 24 h after a second injection, with an interval of 24 h between injections. Rausedil, in a dose of 5 μg/kg, was injected intraperitoneally. The

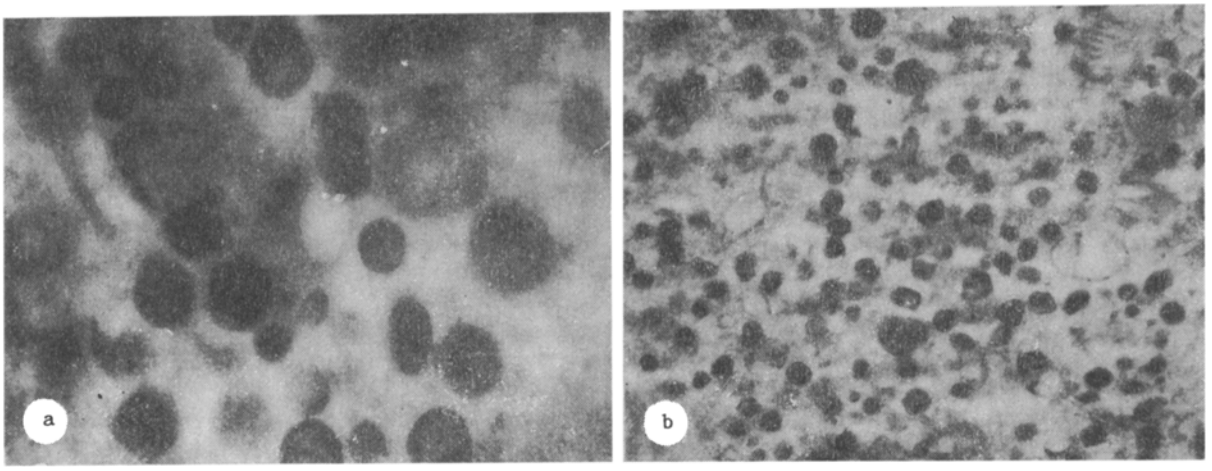


Fig. 1. Reaction of intact rats with sodium molybdate: a) SG, unstained section, 15,000×; b) adrenal chromaffin cells, stained section, 9000×.

Laboratory of Pathomorphology, L. A. Oganesyan Institute of Cardiology, Erevan. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 6, pp. 760-762, June 1986. Original article submitted October 11, 1985.

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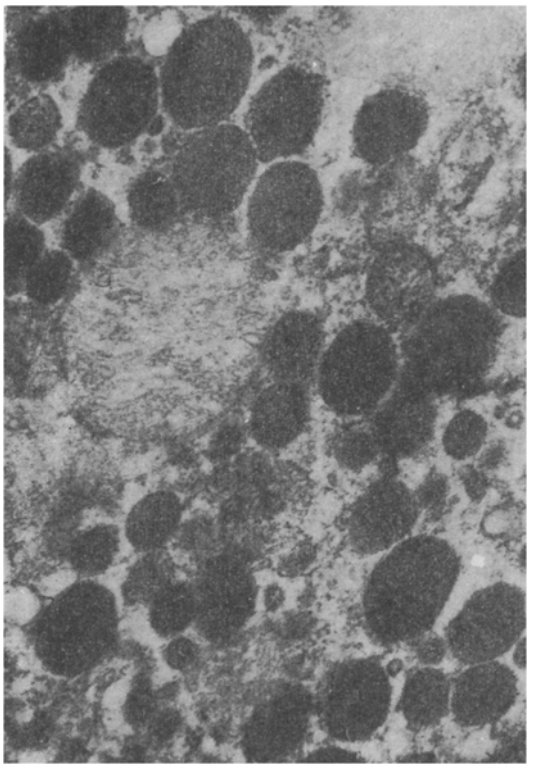

Fig. 2. Increased density of contents and hyperplasia of SG after second injection of adrenalin into intact animals. Stained section. 17,000×.

myocardium was studied 3 h after a single injection and 24 h after a second injection of the drug, with an interval of 3 h between injections. Hemodynamic loading of the heart was produced by constricting the abdominal aorta by Beznak's method in the modification in [3]; the rats were investigated 2 months later. In group 5 the hearts of rats exposed to hemodynamic overloading for 2 months were studied 3 and 24 h after a single injection of rausedil. All the animals were decapitated. Material was fixed in buffered $OsO_4$ solution by Caulfield's method, dehydrated in ethanol, and embedded in a mixture of Epon and Araldite. Catecholamines in SG were detected by the electron-histochemical reaction with potassium bichromate [14] and sodium molybdate [13]. The reaction was verified on chromaffin cells of the adrenal medulla. Protein was determined by the reaction described previously [10]. Ultrathin sections stained with uranyl acetate and lead citrate by Reynolds' method and unstained section were studied in the UÉMV-100 B electron microscope.

## RESULTS

Treatment of the intact rat myocardium with potassium bichromate solution revealed pale SG with no visible residue of any kind inside them. Meanwhile, after the same reaction with tissue of the adrenal medulla, the reaction product was found in granular structures of the chromaffin cells. Fragments of myocardium treated with sodium molybdate solution showed selective staining of SG, which were distinguished by their density and frequently had a distinct outline (Fig. 1a). The corresponding reaction with tissue of the adrenal medulla also was positive (Fig. 1b). The reaction for total protein revealed a uniform, dense precipitate in SG. A single injection of adrenalin caused some increase in the density of SG, and a second injection was followed by an increase in the number and a marked increase in their density (Fig. 2). A single injection of rausedil into intact rats was followed by a slight decrease in the density of the contents of SG. After a second injection of rausedil, SG in some cells appeared less dense, with a finely honeycombed structure. In rats with hemodynamic overloading of the heart, the SG reacted positively with sodium molybdate and weakly positively with potassium bichromate. Injection of rausedil into rats with hemodynamic overloading of the heart caused a decrease in the density of many SG after 3 h, and most SG after 24 h appeared finely granular, they lost their distinct outlines, and became fragmented (Fig. 3).

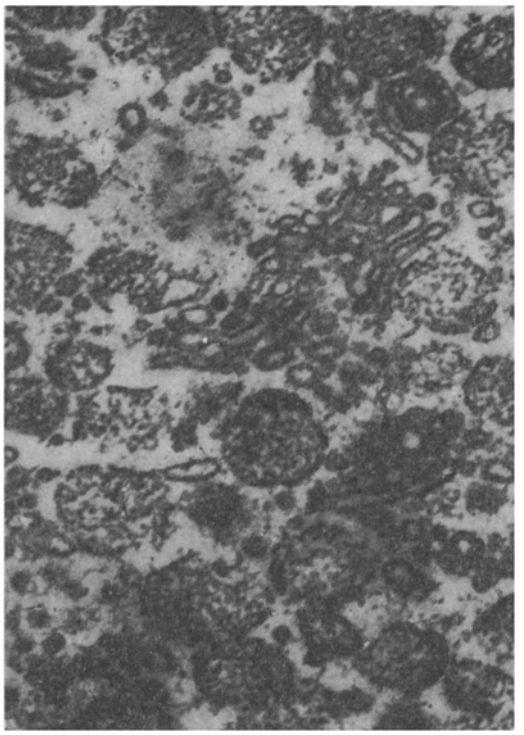

Fig. 3. "Exhausting" effect of rausedil on SG during dynamic overloading of the heart. Stained section. 17,000×.

The electron-microscopic study of SG of the intact myocardium in the search for catecholamines gave inconsistent results. The SG did not react with potassium bichromate, the traditional marker of catecholamines. However, the reaction with sodium molybdate, suggested for the detection of biogenic amines in the pineal gland [13], was positive both in SG and in granules of the adrenal medulla. It can be tentatively suggested that the concentration of catecholamines present in SG is too low to be revealed by potassium bichromate, but reacts positively with sodium molybdate, a more sensitive indicator. Further evidence of the presence of catecholamines in SG was given by the "exhausting" effect of rausedil, which is a sympatholytic, especially if given in two doses. Other investigators [11] have used similar evidence of the presence of catecholamines in SG. Besides catecholamines, the SG also contained protein.

The connection between SG and the injected adrenalin is a more difficult problem to interpret. Judging from data in the literature, labeled catecholamines and their precursors were not incorporated into SG [12]. Exogenous adrenalin may perhaps stimulate accumulation of endogenous catecholamines in SG. Whatever the case, the results of the experiments with adrenalin are evidence that the state of SG depends on the catecholamine levels in the body. Similar results to those now described were obtained by other workers who injected rats with norepinephrine and L-dopa [5]. Meanwhile, there are reports in the literature of negative correlation (admittedly, not statiscally significant) between the catecholamine concentration in the myocardium and the number of SG [6]. It must be pointed out in this collection that the degree of involvement of SG in the general balance of catecholamines entering the myocardium from the blood stream and synthesized in adrenergic nerve endings may be comparatively unimportant. Under conditions of hemodynamic overloading of the heart SG gave a positive reaction with potassium bichromate and sodium molybdate, indicating accumulation of catecholamines in them. Evidence in support of this view is given by well-marked "exhausting" effect after injection of rausedil.

The role of SG in the accumulation and utilization of catecholamines demonstrated above, which does not rule out their participation in the production of a natriuretic hormone [9], suggests that certain atrial cardiomyocytes may be regarded as cells of a diffuse endocrine system, whose role in the regulation of fine cell-tissue interrelations has attracted the close attention of research workers in recent years.

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