Quantitative electron microscopic analysis of the specific granule population of rat atrium

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Received August 27, 1968

The “specific granule” population of right atra of female hooded rats increased with age.

Introduction
It has been established that granules 100 to 450 m\(\mu\) in diameter are present in atrial myocardial cells of vertebrates (Bloom et al. 1961; Jamieson and Palade 1964; Trillo et al. 1966) and also in non-mammalian ventricular myocardial cells (Nayler and Merrillles 1964; Trillo and Bencosme 1965; Trillo et al. 1966). These granules have been considered specific cell organelles different from lysosomes (Jamieson and Palade 1964; Martinez-Palomo and Bencosme 1966b; Trillo et al. 1966). Although these “specific granules” were thought to be a site of catecholamine storage (Bloom et al. 1961; Palade 1961), this possibility was not supported by further investigation (Jamieson and Palade 1964). Attempts have been made to modify the granule population by reserpine (Kisch 1965; Palade 1961), angiotensin (Cote et al. 1965), and adrenal regeneration hypertension (Martinez-Palomo and Bencosme 1966a). The effect of age on the atrial granule population has also been briefly commented upon (Jamieson and Palade 1964), but without quantitative analysis of the data. The present work was undertaken to investigate quantitatively the effect of increase in body weight, as a function of age, on the number of “specific granules” in atrial myocardial cells of female hooded rats.

Materials and Methods
Twenty-two female hooded rats were used in two sets of experiments. In the first, two groups of five rats each were employed; younger rats weighed between 55 and 80 g, whereas older rats weighed between 190 and 215 g (Table 1). In the second experiment, 12 rats weighing approximately 55 g were caged in pairs in a temperature-controlled room and fed Purina Chow. Weekly, the animals were killed in pairs and grouped from I to VI according to the order of sacrifice (Fig. 1).

For electron microscopic studies, tissues were obtained from the anterior wall of the right atrium under ether anesthesia, fixed for 2 h in 5% glutaraldehyde in 0.1 M Sorensen’s phosphate buffer (pH 7.4), post-fixed in buffered osmium tetroxide (pH 7.4), and embedded in Epon 812. In each animal, three Epon blocks were selected for thin sectioning. Sections were doubly stained with uranyl acetate and lead citrate. From each Epon block five photographs 2 \(\times\) 2 in. were taken at a magnification of 2000 to 2060 times with an electron microscope RCA EMU 3D. Photographs were taken using the following criteria: placed in the center was a nucleus no less than 7.5 \(\times\) 2.5 \(\mu\) in size from a longitudinally sectioned myocardial cell with two paranuclear areas (Figs. 2 and 3). Each area studied covered 600 \(\mu^2\) and was termed a unit area. All atrial myocardial “specific granules” present in each unit area were counted on contact prints with a 3.5 \(\times\) magnifier. From each Epon block the first five photographs conforming to the description given above were taken. Fifteen unit areas were counted from each animal. The data obtained were pooled according to group and analyzed statistically.

Results

Experiment I
As shown in Table 1, older rats had a significantly greater variance of atrial myocardial granule population than younger rats. Based on the large sample theorem, analysis of data revealed that the mean number of the “specific granules” per unit area of right atria of older rats was significantly larger than that of younger rats. One unit area from each group is shown in Figs. 2 and 3.

Experiment II
Having established that the granule population of atrial myocardial cells from young rats

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was significantly smaller than that of older rats, we carried out the second experiment to discover the mode of expansion of the granule population in the period of growth between the two weight groups. The body weight of all six groups of rats killed at weekly intervals increased with time (Fig. 1). The rate of growth between groups I and II was noticeably greater than that among groups II to VI. Data on the specific granule populations of the right atria from these rats are summarized in Table II. In spite of a considerable heterogeneity of the variances of the "specific granule" populations of the groups killed at weekly intervals, it is apparent that the mean and variances of the population in cover increased with weight. The correlation coefficient between body weight and mean number of atrial "specific granules" per unit area of atria of the animals studied was 0.8870. For the estimation of significance of the correlation coefficient, \( t = 6.0743 \) and \( P < 0.0005 \).

**Discussion**

The major difficulty in quantitative electron microscopic analysis of the "specific granule" population of rat atrial myocardial cells appears to be in sampling. There is a great variation in the distribution of granules from cell to cell as well as from region to region. To obtain samples as uniform as possible, a restricted area of the right atrium was selected for study. It is evident that in the present work, estimation of the granule population was designed to detect changes mainly in the paranuclear areas. Morphological similarities between "specific granules" and lysosomes and their related structures could be a source of error in counting specific granules. Fortunately, the number of lysosomes and the related organelles is too small to contribute much to the error in counting the specific granules.

This study disclosed that older rats had a significantly larger "specific granule" population per unit area of atrial myocardium both in mean value and variance than younger rats. The second experiment revealed that the expansion of the atrial granule population in the period studied occurred linearly with the increase of body weight, as indicated by the significantly high correlation coefficient. Al-
Figs. 2 and 3. A unit area of atrial myocardium from a younger rat in group I (Fig. 2) and from an older rat in group II (Fig. 3) in the first experiment, × 3300. Approximately 1.65 times larger than the original prints used for counting the specific myocardial granules.
though the number of animals used in experiment II was too small to permit extensive statistical analysis of our data, it seems from Table II that increases in the granule population may occur in step fashion rather than linearly with time. The greatest increase in the granule number per unit area appears to occur between the weekly groups of I and II, as does the body weight.

Acknowledgments

Gratitude is expressed to Dr. M. T. Wasan for statistical consultations, and to Mrs. Marlene Young for technical assistance.


