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Short Contribution

Lack of proportionality between gene dosage and total muscle protein content in the rat heart

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ABSTRACT Counting of isolated cardiomyocytes has demonstrated that their number was 16.8±0.6 10^6 in both ventricles of weanling rats (28 days after birth), growing in litters of four (fast-growing). In rats growing in litters of 16 (slow-growing), the myocyte number was 11.8±0.8 10^6. In the control group (8 sucklings per litter), there were 14.2±10^6 cardiomyocytes. The fast-growing rats had more octoploid cells than slow-growing ones. Considering ploidy and cell number, the total number of myocyte genomes in fast-growing animals was 45% higher than in slow-growing ones. The total content of contractile proteins in fast-growing weanling animals was higher by 28% while sarcoplasmic proteins were 8% higher. This lack of correspondence between the number of myocyte genomes and muscle protein content was even more pronounced at the age of 110 days. The results are compared with the cytophotometric data concerning the lack of correspondence between the total protein content in a myocyte and its DNA amount and chromosome number, i.e., total dosage of the myocyte genes.

KEY WORDS: *tissue growth, heart development, cell polyploidy, gene dosage*

Recently individual variations in myocyte number and ploidy have been described (for review see Brodsky, 1991). The ventricular myocytes in mice and rats are mainly tetraploid and binuclear 2cx2, where c is haploid NA content). However, individual animals differ in percentage of diploid and polyploid cells and even more in terms of the total number of myocytes in heart ventricles. This observation is particularly interesting in view of the considerable polyploidy variation in the human heart. Transplantation of the embryonic heart muscle under the renal capsule of syngeneic adult rat led to the idea of a time-dependent program of polyploidization (Brodsky *et al.*, 1988). However, the extent of polyploidy and the number of complete divisions of myocytes depend on growth conditions. The problem of mechanisms and consequences of myocyte genome variation is important and interesting. In experiments with excessive or insufficient feeding of suckling rats (Kennedy, 1957), heart weight could be modified by the time of weaning. Stereometric calculations demonstrated that the number of ventricular myocytes in weanlings is different (Rakusan *et al.*, 1978). Demonstration of variation in myocardial DNA content led to a similar conclusion (Dowell, 1984; Penney and Caldwell, 1984). Direct counting demonstrated the increase in the number of myocytes in fast-growing weanlings and the increased percentage of octoploid cells in them; the changes persisted in adult animals (Brodsky *et al.*, 1985a).

The level of muscle proteins may be associated with the size of the myocyte genome. Cytophotometric measurements have shown a lack of correspondence between protein content and gene dosage i.e., tetraploid myocytes do not have twice as many and octoploid cells do not have four times as many proteins as diploid cells (Brodsky *et al.*, 1985b). In the present study we separately examined proteins of muscle and connective tissue in heart ventricles of rats which were growing either slow or fast during the first weeks of life: (1) at the day of weaning, i.e., at the final stage of proliferation of the ventricular myocytes, and 2) at day 110 of life, i.e., after about three months of feeding ad libitum after massive growth of cytoplasm in non-dividing myocytes.

Weight kinetics

The weight of rats growing before weaning in litters of four practically does not differ from the weight of control animals growing in litters of eight up to 110 days after birth (Fig. 1). The weight of rats kept in litters of 16 before weaning was lower than that of fast growing rats ($p<0.05$) for all time points used, with the exception of the day of birth and day 110. At day 21 after birth, i.e., at the end of the milk feeding phase, the weight of rats growing in litters of four exceeded that of rats growing in litters of 16 on average by 63%. At day 28 after birth at the time of weaning (when protein content was studied) the difference in weight between fast- and slow-growing animals amounted to about 40% and was statistically significant. At the age of 110 days the difference, equal to 17%, was not statistically significant, but the shape of the curves clearly dem-

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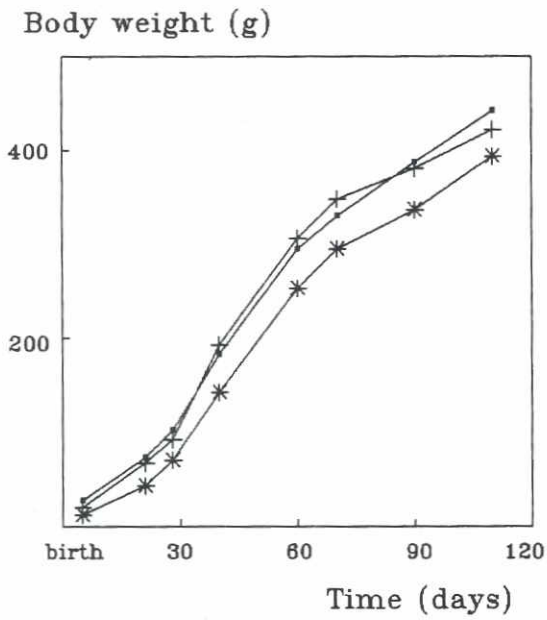

Fig. 1. Growth kinetics of rats kept between birth to weaning in litters of 4 (dots), 8 (crosses), or 16 (asteriscs); *after weaning they were fed ad libitum.*

onstrates that early differences in terms of growth persist in adult animals.

The weight of heart muscle at the age of 28 days in fast-growing weanlings was significantly higher than in the slow-growing ones, the ratio being 1.37; at the age of 110 days the ratio was 1.06.

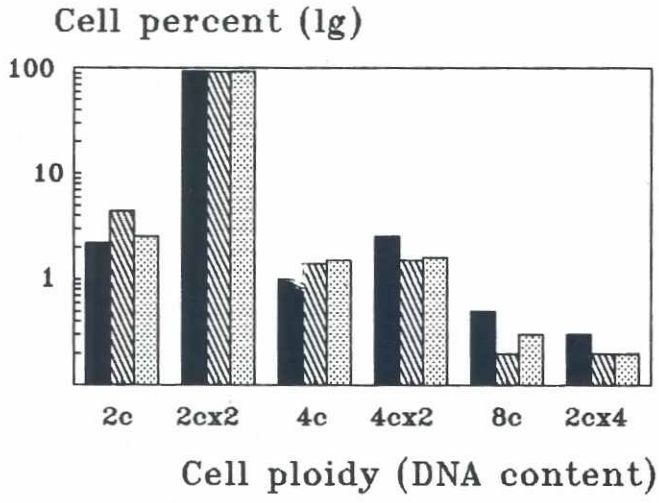

Fig. 2. Percentage of ventricular myocytes of various ploidy classes (c= haploid DNA level) in rats from litters: of 4 (black bars), 8 (hatched bars), or 16 (dotted bars).

Ploidy and the number of myocytes

In fast-growing rats the number of octoploid myocytes (4cx2, 8c, and 2cx4 together) was approximately twice as high as in the slow-growing animals (Fig. 2). Such cells, however, were scarce, whereas the number of binuclear tetraploid cells 2cx2, accounting for more than 90% of all myocytes, did not differ in various groups of rats.

The number of myocytes showed significant differences (Table 1). This number differed on average by 42% between the fast- and slow-growing weanlings and by 16% between the former and the control. The number of cells of each class was calculated from the percentage of myocytes and, correspondingly, we also calculated the total number of genomes in haploid equivalents. The total genome was defined as the sum of genomes in diploid, tetraploid and octoploid cells.

Protein content

The content of total muscle proteins (product of the protein concentration and organ weight) in the left ventricle of fast-growing weanlings was higher than in the slow-growing ones (Fig. 3). However, the level of muscle proteins in the right ventricle was virtually identical. Both the left and the right ventricles in various groups of rats differed greatly in the content of connective tissue proteins.

TABLE 1

THE NUMBER OF GENOMES IN COMBINED LEFT AND RIGHT VENTRICLES OF RATS FROM DIFFERENT LITTERS

Litter	Cell number 10^6	Genome number 10^6c
4	16.8 ± 0.6	68.5 ± 2.5
8	14.2 ± 1.0	56.4 ± 3.9
16	11.8 ± 0.8	47.4 ± 3.2

The non-muscle proteins were mainly responsible for the excess of protein weight in the heart muscle of fast-growing weanlings. By day 110 the concentrations of muscle and connective tissue proteins in the left and right ventricles of rats with initially different growth rates were practically equal. At this time the weight of the heart muscle also was similar, as well as the total content of muscle and other proteins.

The genome-to-protein ratios

Since the number of myocytes and their ploidy were determined in cells isolated from two ventricles, Fig. 4 shows pooled data for myocardial proteins. The ratio of total genomes in myocytes was 1.45, which is close to the ratio of the total weight of myocardial proteins in fast- and slow-growing weanlings. However, this correspondence is only an apparent one. Connective tissue makes a major contribution to the total protein. The ratio of genomes, however, refers only to myocytes. The ratio of protein content was 1.26 for the contractile proteins and 1.08 for the sarcoplasmic ones in weanlings.

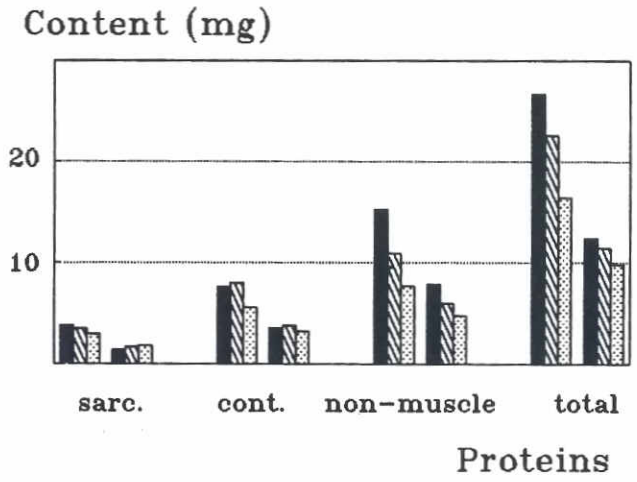

Fig. 3. Level of sarcoplasmic, contractile and connective tissue (non-muscle) proteins, plus total weight of proteins in the left (the first group of columns) and right ventricle of rat weanlings growing in different litters. *For definition of the columns see Fig. 2).*

At day 110 the ratios of muscle protein content were close to 1, whereas the total myocyte genome in formerly fast-growing rats exceeded the size of genome of slow-growing animals by 45%.

The main result of our study is the demonstration of a lack of correspondence between the total myocyte genome in fast- and slow-growing rats on the one hand, and the content of muscle proteins in their heart, on the other. These results do not exclude a possible correlation of one or the other isoform to gene dosage undetected by the method used. The ratio between isomyosins changes a little in weanling rats. Dowell and Martin (1984) determined 6% and 16% for V_3 isoform and 94% and 84% for V_1 isoform in the weanling rats growing 4 or 16 per litter. We do not know the mass ratio as well as the isoform content in adult rats. Concentration of total myofibrillar proteins was similar in fast- and slow-growing rats (Dowell, 1984; Dowell and Martin, 1984), which demonstrated in our opinion no correspondence between these proteins and gene dosage. The relations between the myocyte genome and cell mass were first studied cytologically (Brodsky *et al.*, 1983, 1985b). At the moment of polyploidization, the protein mass of cardiac myocytes corresponds exactly to the gene dosage, as follows from DNA cytophotometry and cytophotometric measurements of proteins. However, soon after the cessation of mitoses, when the myocyte cytoplasm starts to grow, the ratio of protein contents in diploid, tetraploid, octoploid and hexadecaploid myocytes becomes 2:3.3:5:6.3. This ratio of myocyte protein content, which does not correspond to gene dosage, was present in mice at the age of one year, after about 10-fold increase in the protein content in every cell. In rats we found similar ratios in the protein content of di-, tetra- and octoploid myocytes 2:3.5:6.2.

The lack of correlation between the content of muscle proteins and the total myocyte genome has now been noted in measurements of the cell number, whereas percentage of major myocyte classes remained almost unchanged. Therefore, maintenance of the myocardial muscle weight during ontogenesis represents a tissue-specific, rather than a cell-specific, regulation.

By day 110 after birth the heart weight of rats that were growing at different rates during the first four weeks of life was similar. Cytophotometry demonstrates that in adult animals the total protein content per myocyte is also similar Brodsky *et al.*, 1985b). In the present study we have found equal content of muscle and connective tissue proteins. It appears that this similarity of muscle protein content explains similar physiological parameters of the heart in adult rats from groups that were formerly growing at different rates.

Characteristics of the heart in these animals such as speed of contraction and relaxation, coronary blood flow, and functional reserve do not differ (Ostadal *et al.*, 1992). Changes in blood pressure in response to isoproterenol are also similar. However concerning the myocyte genome, there are marked differences. The end of myocyte proliferation corresponds in mice and rats to the day of weaning (Rumyantsev, 1977; Brodsky *et al.*, 1980).

The lack of proportionality between the DNA content (i.e., genome weight) and the amount of muscle protein can be due to the decreased activity of genes coding for muscle proteins (gene dosage compensation effect). Alternatively all the genes are active but the activity is different in various groups of rats or in cells of different ploidy. Finally, the non-proportionality may be realized at the level of protein synthesis.

Until recently, the effect of gene dose compensation was known only in some cells of *Drosophila* and in a few plant species (for review see Epstein, 1986). In the available studies of transcription, translation and other cell properties (about 20 characteristics have been studied for hepatocytes), complete correspondence of the properties to gene dosage has been demonstrated in various polyploid cells (see Brodsky and Uryvaeva, 1985). Cardiac myocytes provide an exception. These cells differ from the others by their intense growth after polyploidization. Mice and rats grow practically throughout life, but the cardiomyocyte genome increases in size only during the first three to four weeks. The total number of

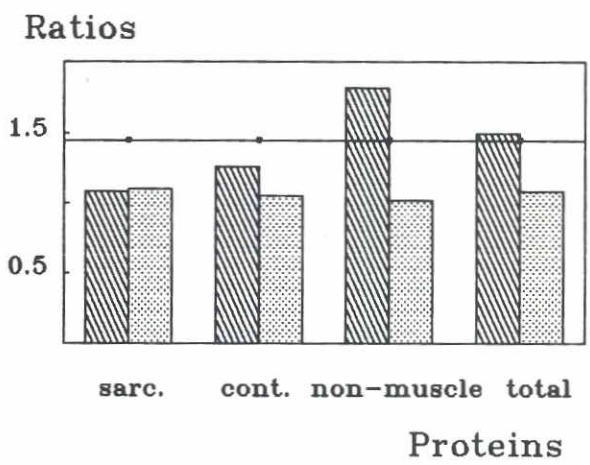

Fig. 4. The ratio of total myocyte genome in rat weanlings growing in litters of 4 and 16 (4:16, solid line) as compared with ratios of different proteins and the ratio of total protein content in the same groups. *Each value corresponds to the two ventricles in sum. Hatched bars= weanling rats, dotted bars= 110-day-old rats.*

genomes appears to be irrelevant for the normal heart function. However, under the conditions of cardiac overload or hyperfunction, excess or deficit of genome may serve as a factor of adaptation of the heart muscle and therefore of the whole organism.

Experimental Procedures

Animals

Newborn Wistar rats were assigned randomly in groups of 4, 8 or 16 to different females and reared in such litters up to day 28. Normally rat females feed 7-8 newborns. After weaning rats were kept in cages in groups of four and fed ad libitum. Heart ventricles of rat males were studied on the day of weaning (28th day after birth) and in adult animals (110 days). Animals were sacrificed by cervical dislocation.

Isolation of cells and counting of myocytes

The heart muscle was fixed by formaldehyde made in phosphate buffer, pH 7.0 (1:9) for no less than two weeks. The central part of the myocardium was than excised (left and right ventricle together) and dissociated into individual cells using 50% KOH (Grabner and Pfitzer, 1974; Kogan *et al.*, 1976). We have adapted this technique for reproducible cell counting (Brodsky *et al.*, 1985b).

We have demonstrated NA and protein conservation in isolated cells (see also Brodsky *et al.*, 1980). Myocytes were counted in suspension using a Fuchs-Rosenthal chamber.

DNA cytophotometry

Smears were prepared on microscope slides from the suspension of isolated myocardial cells. They were stained by Feulgen reaction (hydrolysis in 5 N HCl for 10 min at 37° followed by the treatment with Schiff reagent for 1 h at 20°). A Vickers M86 microdensitometer was used for DNA cytophotometry: objective lens x100, scanning probe 0.4μ, wavelength about 580 nm.

Measurement of protein

For biochemical measurements of protein (Pelouch *et al.*, 1984) the left or right ventricle was isolated, washed with deionized water, cut into small pieces and quickly homogenized. The homogenate was then treated with 0.03 M phosphate buffer, pH 7.4 and centrifuged at 7000 rpm. Sarcoplasmic proteins were measured in the supernatant. The pellet after centrifugation was resuspended in the same buffer with 0.1 M potassium iodide. The supernatant was used to measure contractile proteins. The pellet after this centrifugation contained proteins of the connective tissue and myocardial vessels: collagens of various types, elastin, and glycoproteins.

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