Cardiac-Muscle Hypertrophy

DIFFERENTIATION AND GROWTH OF THE HEART CELL DURING DEVELOPMENT

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An experimental model for the study of the control of tissue growth by cell proliferation (hyperplasia) and cell enlargement (hypertrophy) is proposed. The model is constructed on the basis of the fact that, when DNA replication and cell proliferation cease in cardiac muscle during neonatal development, subsequent growth of the ventricular tissue is due to cell enlargement. The time period during development when this change in growth pattern occurs is documented with the cessation of DNA replication and the synthesis and accumulation of myosin as biochemical parameters. It is suggested that adrenergic innervation of the heart may be the physiological signal that changes the growth pattern of the muscle from hyperplasia to hypertrophy.

When exposed to an increased work load, heart-muscle cells of the adult respond by increasing the synthesis of their contractile proteins and enlarge or undergo compensatory hypertrophy to accommodate these new contractile elements. Factors responsible for the initiation of compensatory cardiac hypertrophy and the mechanism by which an increased functional demand is converted into such a physiological event are unknown. In seeking to identify these factors, several experimental animal systems, including pressure and volume overload, have been used to produce compensatory cardiac hypertrophy (Fanburg, 1970; Rabinowitz & Zak, 1972; Rabinowitz, 1974; Morkin, 1974).

Growth of the heart during early development is by cell division and proliferation (hyperplasia) (Goss, 1966). In cardiac muscle of the rat, semi-conservative DNA replication, and hence growth by hyperplasia, essentially ceases by the middle of the third week of postnatal development (Claycomb, 1975, 1976*a*). Increase in muscle mass thereafter must be due, therefore, to enlargement (hypertrophy) of these pre-existing cells and accumulation of contractile proteins. The present paper reports experiments that document the time period during development when the growth pattern changes from hyperplasia to hypertrophy. I suggest that the developing heart offers an ideal natural model system for studies of the factors that control cardiac-muscle growth by cell hypertrophy.

Experimental

[³H]Phenylalanine (specific radioactivity 16.1 Ci/mmol) and [¹⁴C]phenylalanine (specific radioactivity 464 mCi/mmol) were from New England Nuclear

Corp., Boston, MA, U.S.A. Timed pregnant rats were obtained from Holtzman (Madison, WI, U.S.A.) on day 14 of gestation. They were housed in individual cages and maintained on water and standard laboratory chow *ad libitum*. Neonatal rats were raised in litters of ten. The source of all other chemicals and materials was as previously described (Claycomb, 1975, 1976*a,b*).

Cellular protein and myosin content and cellular uptake and incorporation of [³H]phenylalanine into protein and into myosin were determined as previously described (Claycomb, 1976*a*).

Results and Discussion

Myosin content and synthesis were measured at various times during development to determine when the contractile proteins begin to accumulate. Since the majority of proteins in the muscle cell are contained in the contractile elements, total cardiac-muscle protein was also measured to compare changes in myosin with changes in other contractile proteins. Incorporation of [³H]phenylalanine into myosin and into total muscle protein was used as a measure of myosin and protein synthesis respectively. To ensure that the actual rate of incorporation of [³H]phenylalanine was being measured, the kinetics of incorporation were first determined (Fig. 1). Cellular uptake of [³H]phenylalanine after a single subcutaneous injection is represented in Fig. 1 as acid-soluble radioactivity. [³H]Phenylalanine rapidly penetrates the muscle cell and reaches a maximum concentration 5 min after administration. Incorporation of [³H]phenylalanine into muscle protein and into myosin of newborn animals is linear with time for at least 30 min (Fig. 1). A similar time course of incorporation is also observed in 22-day-old and

Fig. 1. Kinetics of cellular uptake and incorporation of [3 H]phenylalanine into cardiac muscle protein and into myosin

Two-day-old neonatal rats were injected subcutaneously with [3 H]phenylalanine ($1 \mu\text{Ci/g}$ body wt) and killed at the times indicated. Cellular uptake (Δ — Δ) and incorporation of [3 H]phenylalanine into total cardiac-muscle protein ($\circ$ — $\circ$) and into myosin ($\bullet$ — $\bullet$) were determined as previously described (Claycomb, 1976b). Ventricular cardiac muscle from ten rats was pooled for each determination.

adult animals. In subsequent experiments a 20 min labelling period was chosen so that the actual rate of incorporation could be determined in animals of different ages.

Cellular protein and myosin content, and cellular uptake and incorporation of [3 H]phenylalanine into protein and into myosin during postnatal development, are shown in Fig. 2. Muscle protein and myosin content, expressed on a DNA or per cell basis, remain relatively constant during the first 2 weeks of postnatal development and begin to increase by the end of the third week (Fig. 2a). Cellular uptake and incorporation of [3 H]phenylalanine into myosin and into protein show a similar temporal pattern (Fig. 2b).

The wet wt. of ventricular cardiac muscle of the 18-day-old rat is $127.8 \pm 3.2 \text{ mg}$; it is $784 \pm 30 \text{ mg}$ in the adult rat with a body wt. of approx. 260 g. Thus the weight of this tissue increases approx. 6-fold and the cellular protein and myosin contents increase 4-fold (Fig. 2a) during the developmental period when the cells stop dividing (Claycomb, 1975) and the period when the animal attains its adult size. These non-dividing cells must enlarge, therefore, to accommodate this increase in contractile protein content (Fig. 2). Histological measurements show that cardiac-muscle cells of the adult are approx. 10-fold larger than those of the 1-day-old rat (Sasaki *et al.*,

Fig. 2. Cellular protein and myosin content of cardiac muscle (a) and cellular uptake and incorporation of [3 H]phenylalanine into cardiac-muscle protein and into myosin (b) during postnatal development

Myosin content and synthesis must be expressed on a DNA or per cell basis to use this parameter as a valid estimate of cellular hypertrophy. To express myosin content and incorporation of [3 H]phenylalanine into myosin as per unit of DNA, the recovery of myosin during extraction must be determined. Recovery was determined by adding a known amount of ^{14}C -labelled cardiac-muscle myosin (prepared by injecting neonatal rats with [^{14}C]phenylalanine and then isolating radioactive cardiac-muscle myosin) to each tissue homogenate immediately before the myosin was extracted. In this way recovery of myosin during each extraction can be determined. If the DNA content of the homogenate is measured, the myosin content and incorporation of [3 H]phenylalanine into myosin on a DNA or per cell basis can be determined. The concentration of total cardiac-muscle protein and myosin in samples of fresh tissue, and uptake and incorporation of [3 H]phenylalanine, were determined as previously described (Claycomb, 1976b). Depending on the age of the animal, between two and 12 hearts were pooled for each determination. Adults were females that weighed approx. 250 g. (a) Protein content, $\circ$ — $\circ$; myosin content, $\bullet$ — $\bullet$. (b) [3 H]Phenylalanine incorporation into protein, $\circ$ — $\circ$; [3 H]phenylalanine incorporation into myosin, $\bullet$ — $\bullet$; cellular uptake (acid-soluble [3 H]phenylalanine radioactivity), Δ — Δ .

1968). The observations reported in the present paper suggest an experimental animal system that can be used to study cardiac-muscle cell hypertrophy. The

synthesis and accumulation of myosin during development can be used to determine the degree of this cellular hypertrophy.

What is the physiological signal for the heart to change its growth pattern from hyperplasia to hypertrophy? Evidence suggests that functional adrenergic innervation of cardiac muscle inhibits DNA replication and promotes terminal-cell differentiation and that noradrenaline and cyclic AMP serve as chemical mediators in this response (Claycomb, 1976b). Functional adrenergic innervation of cardiac muscle may be acting at the physiological level, as well as at the biochemical level, as a signal to change the growth pattern of this tissue from cell division to cell enlargement. Cardiac hypertrophy in the adult is in response to an increased functional demand placed on the heart (Fanburg, 1970; Rabinowitz & Zak, 1972; Rabinowitz, 1974; Morkin, 1974). The stimulus for the muscle cells to hypertrophy in the neonatal animal could be the same, with the increase in functional demand originating from an increase in adrenergic-nerve activity. The heart rate of the laboratory rat increases from 300 beats/min to approx. 500–600 beats/min during the first 3 weeks after birth (Wekstein, 1965; Adolph, 1967). This increase in heart rate is due exclusively to the adrenergic sympathetic nervous system (Wekstein, 1965; Adolph, 1967). Apparently this is a mechanism that the autonomic nervous system uses to adjust the neonatal animal to the increased haemodynamic load encountered after birth. I suggest that, as a response

to this increased functional demand, the muscle cells cease dividing and increase their production of contractile elements, and therefore subsequent growth of the organ is by cell hypertrophy. The size of an organ is largely determined by the work it has to do. The physiological mechanisms that regulate function apparently also control growth. The developing heart and hypertrophy of the cardiac-muscle cell offer an excellent example of this biological concept.

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