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NUCLEIC ACID CHANGES AND NUCLEAR RNA POLYMERASE ACTIVITY
IN EXPERIMENTAL CARDIAC HYPERTROPHY¹

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The development of myocardial hypertrophy appears to be a fundamental adaptive mechanism of the heart in response to an increased work load. The precise manner by which a mechanical stimulus such as pressure or volume overload is translated into a well-ordered biochemical and cellular response is however, as yet, not clear. During the last few years, our laboratory has been studying the biochemical and cellular phenomena associated with cardiac hypertrophy produced by acute supra-valvar aortic stenosis in rats.

This paper deals chiefly with the changes in the nucleic acids and the DNA-dependent RNA polymerase of heart muscle during acutely induced cardiac hypertrophy. Our standard system for producing cardiac hypertrophy consists of banding of the ascending aorta of a mature female Sprague-Dawley rat by means of a specially designed silver clip. In this model system, we obtain increases in heart weight and in DNA and RNA content of the left ventricle of the order of 20 % to 50 % within 48 hours after surgery. Sham-operated litter mates serve as controls. The details of the surgery and the various methods used in the estimation of hypertrophy and the nucleic acid content of the myocardium have been published recently by Nair et al. (1968).

CHANGES IN RNA CONTENT OF THE LEFT VENTRICLE

There is a very early increase in the RNA content of the heart (micrograms per total ventricle) and in the RNA "concentration" (micrograms per milligram wet weight) of the left ventricular muscle. These changes occur within 24 hours after aortic banding (Figure 1). On the second day, maximal increases in RNA concentration (30 %) and total ventricular RNA

(65 %) are seen over that of sham-operated controls. For about two weeks thereafter, these changes maintain a plateau value. A few months later there is a definite tendency for the RNA concentration to fall (Nair *et. al.*, 1969). In Figure 2, we have plotted the percentage of increase in heart weight over controls versus the percentage of increase in RNA. Note that the hearts that manifested the greatest degree of hypertrophy had also the greatest increment in RNA content.

Fig. 2

The radioactivity profile and the optical density pattern of the left ventricular RNA synthesized during a one-hour pulse of ^{32}P (12 to 24 hours after aortic constriction) was analyzed by sucrose density gradient centrifugation (Koide and Rabinowitz, 1969). In Figure 3, we note that the radioactive profile does not quite overlie the optical density pattern. There appears to be a region of elevated specific radioactivity in the zone to the left of the 28s peak. This may be a reflection of the synthesis of ribosomal-precursor RNA. In our experience as well as that of Posner and Fanburg (1968) the RNA in the 4s to 18s region remains highly labeled over the next few hours. It has been suggested that this may be a reflection of the synthesis of messenger RNA for myosin. These studies need further confirmation, e.g., by RNA-DNA hybridization.

Fig. 3

Of considerable interest is the demonstration by Koide and Rabinowitz (1969), that there is an early expansion of the uridine nucleotide pool during cardiac hypertrophy. By contrast the specific activity of the adenine pool does not change significantly. Whether these changes in the uridine nucleotide pool represent an early need for specific uridine nucleotides such as UDPGlucose (which is required in glycogen

synthesis) or are related to an increased utilization of uridine nucleotide for RNA synthesis remain conjectural for the present time.

The base composition of the RNA synthesized during cardiac hypertrophy does not show any appreciable changes from those of sham-operated controls. In both cases, the RNA base composition is as follows: adenine 19.7 ± 2.8 , guanine 32.2 ± 0.3 , uridine 17.0 ± 2.5 , cytosine 31.2 ± 0.5 . Nair et al. (1959) found essentially similar values for RNA synthesized by an in vitro system using purified nuclei from normal and hypertrophied hearts.

CHANGES IN RNA POLYMERASE ACTIVITY

It is now known from experimental evidence in bacterial and mammalian systems that DNA-dependent RNA polymerase is the key enzyme that regulates genetic transcription. Because changes in protein synthesis may be due to alterations in the activity of this enzyme, we prepared pure nuclear fractions from heart muscle by a modification of the method of Widnell and Tata (1964) for liver cells (Nair et al., 1968).

The constituents of the assay system are as follows: System I (low ionic strength conditions). The 0.5 ml incubation mixture consists of 50 μ moles Tris-HCl buffer, pH 7.5, 2.5 μ moles $MgCl_2$ 10 μ moles freshly neutralized glutathione, 5 μ moles phosphoenolpyruvate (Sigma), 10 μ g of pyruvate kinase (Sigma), 3 μ moles of NaF, 0.4 μ moles each of GTP, CTP, and ATP (Sigma), 0.024 μ mole of UTP- $\alpha^{32}P$ and 0.1 ml of nuclear suspension containing 100 to 200 μ g DNA. The specific radioactivity of the UTP is generally 200-300 μ c/ μ mole. In System II (high ionic strength conditions) the incubation mixture is essentially similar to the one above except that

it contains in addition, 300 μ moles of $(\text{NH}_4)_2\text{SO}_4$ adjusted to pH 7.5 with NH_4OH , and Mn^{++} replaces the Mg^{++} ion in the assay. Incubations are carried out in duplicate or triplicate for 5 minutes at 37°C . The details of the extraction of RNA and the counting procedure are given in an earlier paper (Nair et al., 1967).

Figure 4 shows the profile of the changes in RNA polymerase activity from heart muscle nuclei following aortic banding. An early increase is noted within 12 hours. Elsewhere in this volume Schrieber et al. noted similar increases in activity of this enzyme within 1 hour following cardiac overloading in a perfused preparation. Peak activity with a 40 % increase over that of controls is seen in 2 days following aortic narrowing. Thereafter, the enzyme activity gradually diminishes and tends to return to normal levels by the end of the second week.

The base composition of the RNA synthesized in System I is similar to ribosomal RNA (58 % GC content), and in System II is DNA-like RNA. These base composition studies were made by the method of Josse et al. (1961) and have been reported in detail earlier (Nair et al., 1967).

CHANGES IN DNA CONTENT DURING HYPERTROPHY

We now turn our attention to the changes in DNA content of the left ventricle during cardiac hypertrophy. Since mature heart muscle is a well-differentiated tissue we should not expect any mitotic activity in muscle cells. Yet, in Figure 5, we see that the total DNA content of the myocardium does increase during cardiac hypertrophy and the DNA "concentration" per milligram wet weight does not decrease with increasing muscle mass in the early stages. Thus "dilution" of a fixed amount of DNA

does not occur. The theoretical causes that might produce changes in DNA content may be listed under: (1) an increase in polyploidy of nuclei ($2n$ = diploid; $4n$, $8n$ or $16n$ = polyploid), (2) mitosis, (3) karyokinesis without cytokinesis, (4) polyteny, (5) infiltration by round cells from the blood stream, and (6) increase in mitochondrial DNA.

It is important to realize that data pertaining to changes in DNA content of the heart should be analyzed with regard to the maturity of the animal and the duration and intensity of the hypertrophic process. Our data refer to the changes in DNA content of the hearts of mature Sprague-Dawley rats during the acute phase of cardiac hypertrophy. These changes are shown graphically in Figure 5. It is evident that the DNA concentration (micrograms DNA per milligram wet weight) in the heart does not fall as the muscle mass increases and that the total DNA content of the heart is, therefore, increased.

The results of a detailed study of the different contributory factors that could give rise to an increase in myocardial DNA content have been published by Grove et al. (1969) from our laboratory. We have found no evidence for infiltration by inflammatory cells. A survey of the frequency of polyploidy, using the microspectrophotometric method of Swift and Rasch (1956), indicates that although the degree of polyploidy increases from about 2 % to 5 % in muscle cells, this magnitude of change could hardly account for more than 1 % to 2 % of the increment in DNA content.

Mitotic figures were seldom seen in the mature normal heart of rats. In the acutely hypertrophying heart however, it was possible to identify mitotic figures. If one defines the mitotic index as the number of mitoses per 10,000 nuclei, the mitotic index in the hypertrophied heart

was on the average about tenfold as high as that in the controls. Extensive survey of the histological sections failed to reveal unequivocal evidence for mitotic activity in a clearly identifiable muscle cell. We have confirmed this by autoradiography (Grove et al., 1969). Cellular proliferation appears to be restricted to the connective tissue cells, the endothelial cells of blood vessels and other "nonmuscular" cell elements (Figure 6). These are also the findings of Crane and Dutta (1963), Meerson and Alekhina (1967) and Morkin and Ashford (1968). Thus, during acutely induced cardiac enlargement by an imposed work overload the muscle cells increase in mass, without proliferation (hypertrophy) whereas the nonmuscular cells undergo proliferation (hyperplasia). This is in accord with the generally well known biological phenomenon that well differentiated cells do not undergo mitosis after reaching maturity and that less differentiated cells usually respond to the process of growth by hyperplasia.

We have made some preliminary studies on the relative contribution of mitochondrial DNA to the net increase in DNA content of the heart during cardiac hypertrophy. Although it is highly probable that mitochondrial DNA is replicating at the early stage of hypertrophy, the net contribution of this particular species of DNA to the mass of DNA can be regarded as negligible.

Estimations of the hydroxyproline concentration in heart muscle during cardiac hypertrophy show an initial decrease on the second day followed by a rise to above control values by the eleventh day. The total hydroxyproline content (micrograms per grams heart) also increased by the

Fig. 6

eleventh day because of the total increase in cell mass. These studies have been reported in detail by Grove et al. (1969) and further substantiate the data concerning increase in myocardial content.

DISCUSSION AND SUMMARY

In our studies, we have clearly shown that one of the earliest manifestations of cardiac hypertrophy at the biochemical level is an increase in RNA synthesis. The increase is proportional to the degree of hypertrophy during the acute period. We have also shown in purified nuclear preparations that the activity of DNA-dependent RNA polymerase is increased within 12 hours of banding. Schreiber et al. have reported elsewhere in this symposium similar results in perfused hearts subjected to a work overload. It is not yet known whether the increased activity of this key enzyme is due to: (a) activation of the enzyme, (b) removal of an inhibitor, (c) derepression of the DNA template, making new sites available for RNA transcription, (d) synthesis of new units of RNA polymerase. Studies with various inhibitors of the enzyme system are in progress in our laboratory with a view to elaborating the precise mechanism by which enzyme activation takes place.

A detailed biochemical and cellular study of the organization of growth during myocardial hypertrophy has shown that the DNA content of the heart does increase with growth but that in the mature animal nearly all of the DNA increase can be accounted for by the proliferation of the connective tissue cells and other "nonmuscular" elements such as the endothelium. Thus, in conformation with the general observations on well differentiated tissues, heart muscle cells enlarge by hypertrophic growth.

whereas the rest of the cells which are less well differentiated undergo hyperplasia. In the immature heart, where less differentiated muscle cells exist, one can see evidence for mitotic activity (Klinge and Stocker, 1968) but in the mature rat heart we have seen only a very rare cell that shows any evidence for mitosis (Grove et al., 1969). Autoradiographic studies using tritiated thymidine have confirmed the view that the bulk of the increase in DNA content is from cells other than heart muscle. The contribution by polyploidy, binucleate cell formation and by increase in mitochondrial DNA to the total DNA content appears to be negligible.

An unresolved problem is the identity of the cells demonstrating an increase in RNA polymerase activity. Attempts to separate muscle cell nuclei from connective tissue cell nuclei by sucrose density gradient centrifugation have not been successful in our laboratory. Yet another intriguing problem is whether there are embryonic muscle cells in the mature heart which retain the ability to undergo cell differentiation and multiplication by cell division. The ultimate problem, of course, is to understand how a mechanical stimulus is translated into a biochemical response--a problem fundamental to many biological systems.

REFERENCES

Crane, W. A. J., and Dutta, L. P. (1963). J. Pathol. Bacteriol. 86, 83.

Grove, D., Nair, K. G., and Zak, R. (1969a). Circulation Res. 25, 463.

Grove, D., Zak, R., Nair, K. G., and Aschenbrenner, V. (1969b).

Circulation Res. 25, 473.

Josse, J., Kaiser, A. D., and Kornberg, A. (1961). J. Biol. Chem. 236, 864.

Klinge, O., and Stocker, E. (1968). Experientia 24, 167.

Koide, T., and Rabinowitz, M. (1969). Circulation Res. 24, 9.

Meerson, F. Z., and Alekhina, G. M. (1967). Dokl. Akad. Sci. S.S.R.

173, 122.

Morkin, E., and Ashford, T. P. (1968). Am. J. Physiol. 215, 1409.

Nair, K. G., Cutilletta, A. F., Zak, R., Koide, T., and Rabinowitz, M.

(1968). Circulation Res. 23, 451.

Nair, K. G., Rabinowitz, M., and Tu, M. (1967). Biochemistry 6, 1898.

Swift, H., and Rasch, E. (1956). In "Physical Techniques in Biology

Research" (G. Oster and A. W. Pollister, eds.), p. 354. Academic

Press, New York.

Schreiber, S. S. (1969). This Symposium. p.

Widnell, C. C., and Tata, J. R. (1964). Biochemical J. 92, 313.

FIGURE LEGENDS

Figure 1. Changes in RNA concentration and total ventricular RNA at 1 to 12 days after surgery in banded rats (solid circles) and sham-operated rats (open circles). * $P < 0.005$; + $P < 0.05$; x $P < 0.20$.

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Figure 2. Relation between the percentage of increment in RNA concentration and in heart weight after aortic banding. Data are expressed as percentage of increase over sham-operated controls. Determinations were made 7 to 8 days after banding. Open circles = changes in RNA concentration and solid circles = changes in total Ventricular RNA.

(Reproduced by kind permission from Circulation Res. Nair et al., 1968.)

Figure 3. Pattern of labeling of myocardial RNA with ^{32}P . Sucrose density gradient profile of RNA 1 hour after labeling (1 mc ^{32}P per rat). Optical density at 260 m μ (solid line), counts per minute (open circles), and specific radioactivity (x's). (Reproduced by kind permission from Circulation Res. Koide and Rabinowitz, 1969.)

Figure 4. Time course of changes in nuclear RNA polymerase nuclear RNA activity following aortic banding. Each point represents the results from duplicate assays using nuclei from two pooled hearts. Data are expressed as percentage of changes over values obtained from sham-operated controls. (Reproduced by kind permission from Circulation Res. Nair et al., 1968.)

Figure 5. Relation of changes in DNA concentration, and total ventricular DNA, to changes in heart weight after aortic constriction. Data are expressed as percentage of increase over sham-operated controls. Determinations were made 2 to 12 days after banding. The slopes of the lines were calculated by the method of least squares.

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Figure 6. Connective tissue nuclei labeled by autoradiography with ^3H -thymidine. Labeling of muscle cell nuclei was extremely rare.

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Figure 1.

Figure 2

Figure 4

RNA POLYMERASE ACTIVITY AFTER AORTIC BANDING

Figure 1

Figure 5

Figure 7.