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Effect of Calcium on RNA and Protein Synthesis in the Hypertrophied Myocardium (39319)

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RNA and protein synthesis are increased in the myocardium of animals subjected to experimental aortic stenosis (1-3). These processes appear to be initiated by an increase in endocardial tension; however, the mechanism by which an increase in tension is able to affect macromolecular synthesis is as yet unknown. It has been well established that the contractile process and the resultant development of tension in muscles involve Ca^{2+} flux (4-6) and that this ion also has been noted to enhance both RNA and protein synthesis (7-10). In view of these observations, it is of interest to determine if the enhancement of RNA and protein synthesis in the hypertrophied heart is related to the effect of Ca^{2+}.

Methods. Sprague-Dawley rats, weighing 220-270 g, were maintained on food and water *ad libitum*. Aortic constriction was performed with a size-19 needle (1.0-mm o.d.) by the method of Florini (2). Surgery in the sham group (control) was similar except that the thread was loosely tied around the descending aorta. Seven days after surgery the heart was excised and weighed after removal of the atria and aorta. The left ventricle was sliced and 120-150 mg of tissue was incubated in 5-ml Krebs Ringer phosphate buffer, pH 7.4, in the presence or absence of 1.5 m*M* Ca^{2+}. The incubation buffer contained 5.6 m*M* glucose, all the amino acids at normal serum concentration (11), and the radioactive precursors [U-^{14}C]leucine (0.5 μCi/ml) and [5,6-^{3}H]uridine (1.0 μCi/ml).

After the incubation period the slices were rinsed five times in the incubation buffer prior to homogenization. Proteins from the 600*g* (myofibrillar) pellet and the 105,000*g* (sarcoplasm) soluble fraction were precipitated with an equal volume of 20% trichloroacetic acid (TCA), washed, and assayed for radioactivity (14, 15) and protein concentration (12). RNA was extracted from the 600*g* fraction by an incubation for 60 min at 37° in 0.3 *M* KOH according to the method of Fleck and Munro (13), and assayed for radioactivity (15) and total RNA as described by Mejbaum (14).

Ca^{2+} levels were measured in left ventricles by digestion of 100 mg of tissue in 1:3 70% perchloric acid-30% hydrogen peroxide for 4 hr at 70°. The digestion mixture was diluted to 2.0 ml with 0.4 ml of 5% lanthanum oxide and glass double-distilled water, and assayed in Perkin Elmer atomic absorption spectrophotometer at 422.7 nm.

Results. The presence of hypertrophy was determined by assessment of the heart weight to body weight ratio which was $3.13 \pm 0.17 \times 10^{-3}$ (mean ± SD) in the hypertrophy group 7 days postsurgery as compared to $2.74 \pm 0.18 \times 10^{-3}$ (mean ± SD) in the sham ($P < 0.0025$). In further support of the development of hypertrophy is the fact that RNA concentrations were also elevated in the aortic constricted group [557 ± 54 μg/ht (mean ± SD) vs 353 ± 35 μg/ht (mean ± SD) ($P < 0.0005$)].

Although heterogenous RNA decreased after a 3-hr incubation period in both groups, in the absence of Ca^{2+} RNA was lower in the sham than in the hypertrophy group (Fig. 1). In the presence of Ca^{2+} RNA did not decrease as rapidly in the sham group, and the levels of RNA were similar to the hypertrophied group (Fig. 1).

RNA synthesis in the hypertrophy group was essentially the same as the sham (Fig. 2). Although the incorporation of [5,6-^{3}H]uridine into RNA was increased by 1.5 m*M* Ca^{2+} in both groups, no proportionate difference in the Ca^{2+} enhancement could be observed (Fig. 2). The rate of incorporation of labeled uridine into RNA in both the sham and hypertrophy group was linear and essentially the same over the entire 4-hr incubation period as seen in Fig. 2.

Protein synthesis was increased 1.5- to

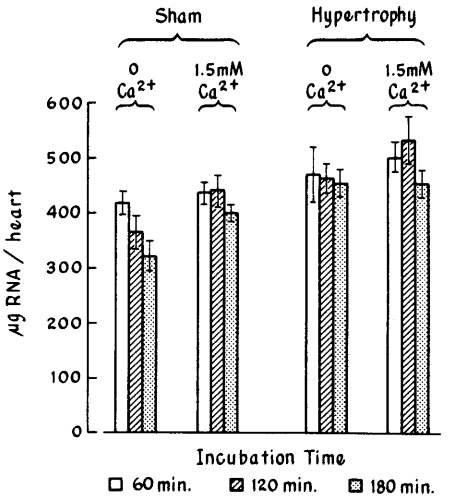

FIG. 1. A bar graph of changes in nuclear RNA concentration in slices of sham and hypertrophied hearts during a 3-hr incubation. Data are based on six experiments and expressed as total micrograms of RNA per heart ± SD.

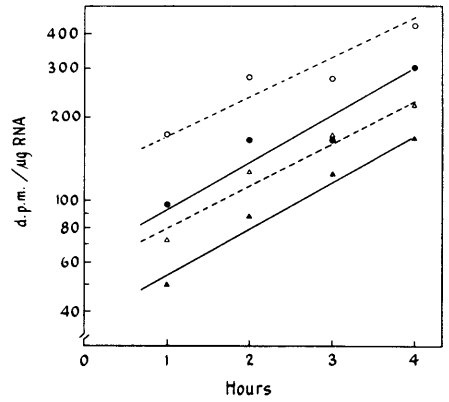

FIG. 2. A logarithmic plot of the rate of synthesis of RNA in sham- and hypertrophy-operated animals. ●——●, ○——○, sham-operated animals; ▲——▲, △——△, aortic-constricted animals; solid symbols no Ca^{2+}, open symbols 1.5 m*M* Ca^{2+}. Data are based on six experiments and are expressed as dpm/µg RNA.

2.5-fold in left ventricular slices from the hypertrophied hearts, as shown in Figs. 3–6. The addition of Ca^{2+} to the incubation buffer stimulated the rate of incorporation of [^{14}C]leucine into protein in both groups. When the incubation period was extended for 4 hr, the rate of myofibrillar-nuclear protein synthesis was decreasing after 3 hr in both the sham and hypertrophied groups (Fig. 3). A logarithmic plot of these data indicated that the rate of synthesis of protein followed first order kinetics (Fig. 4) with a 2.8 greater rate of synthesis in the hypertrophied group. During the first 2 hr Ca^{2+} stimulated more markedly the incorporation of leucine into protein in the sham group (Fig. 4).

The 105,000*g* supernatant proteins in the hypertrophy group also were labeled at a faster rate than in the sham group (Fig. 5). In constrast to the myofibrillar proteins, the soluble proteins in the sham group did not appear to slow down after a 3-hr incubation period. Both groups exhibited an exponential rate of soluble protein synthesis (Fig. 6), and the stimulation of soluble protein synthesis was initially more markedly enhanced by Ca^{2+} in the sham group.

The uptake of [^{14}C]leucine and [^{3}H]uridine into the TCA-soluble fraction over the 4-hr incubation period was not significantly different between the sham-operated and

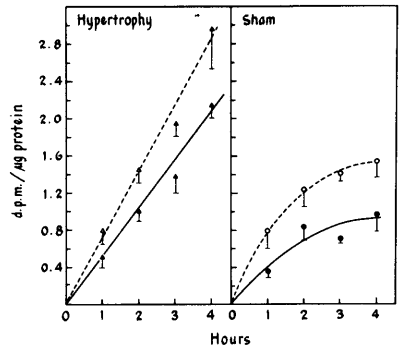

FIG. 3. The rate of incorporation of [^{14}C]leucine into nuclear myofibrillar proteins of sham- and hypertrophied-operated animals. Legend to figure is described in Fig. 2. Data are based on mean of six experiments and expressed as dpm/µg protein.

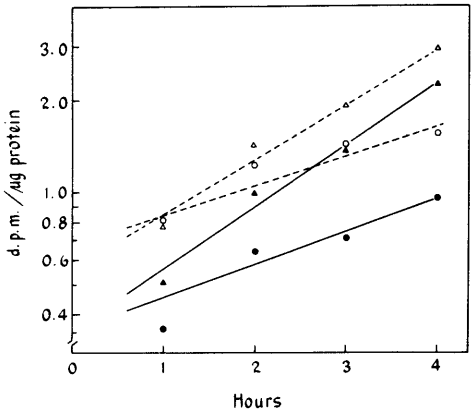

FIG. 4. A logarithmic plot of Fig. 3.

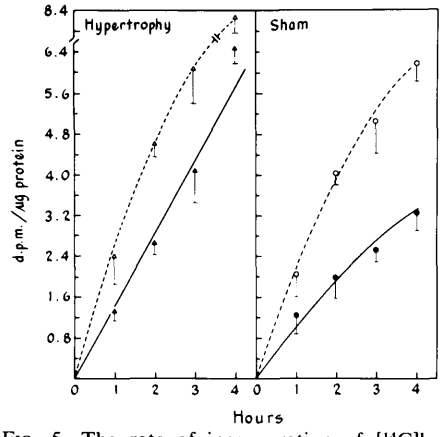

FIG. 5. The rate of incorporation of [^{14}C]leucine into soluble proteins of sham- and hypertrophied-operated animals. Legend to figure is described in Fig. 2. Data are based on mean of six experiments and expressed as dpm/μg protein.

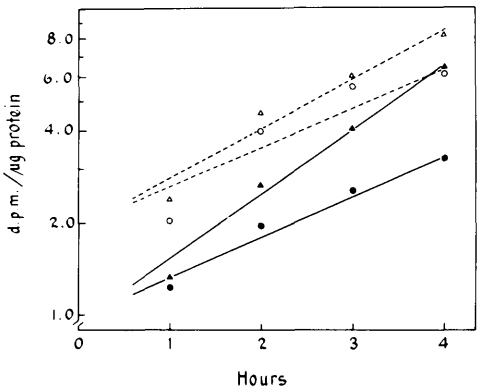

FIG. 6. A logarithmic plot of Fig. 5.

the hypertrophied hearts, as shown in Fig. 7. The presence of Ca^{2+} in the media did not enhance the appearance of RNA and protein precursors in the tissue (Fig. 7). Of interest is the observation that the concentration of Ca^{2+} in the left ventricle was significantly higher 4.8 ± 0.4 mg of Ca^{2+}/100 g wet wt (mean ± SD) in the nonincubated tissue of the hypertrophied hearts compared to 3.0 ± 0.6 mg Ca^{2+}/100 g wet wt (mean ± SD) in the sham hearts ($P < 0.0005$).

Discussion. These studies indicate that the rate of synthesis of RNA and of proteins in myocardial slices from rats subjected to 7 days of experimental aortic outflow obstruction are similar to *in vivo* experiments (1–3). The data demonstrate an enhanced rate of protein but not RNA synthesis at this time interval.

It was noted that protein synthesis in both the sham and hypertrophied groups was stimulated by the presence of exogenous calcium. However, the rate of soluble protein synthesis in the hypertrophied group was proportionately no greater than in the sham. Our observations, as well as others (15–18), indicate that endogenous calcium is elevated in hypertrophy hearts. It is possible that the elevation of intracellular Ca^{2+} may be responsible for the increased incorporation of labeled amino acid into protein (19) in the hypertrophied myocardium.

Another mechanism which may enhance protein synthesis is an increase in concentration of RNA (1, 20). This RNA may also have a long half-life since protein synthesis was not diminished in the hypertrophied group after 7 days. The elevated levels of RNA in the hypertrophied group may be also due to an initial increased rate of synthesis of RNA (1, 20) or decreased degradation of RNA. The initial increased rate of synthesis of RNA may have been stimulated by an increased level of endogenous Ca^{2+} (8, 19) during the early development of hypertrophy.

The results of this study also indicate that *in vitro* the level of RNA in the hypertrophy group did not decrease as rapidly as the sham (Fig. 1), and this decrease can be replicated in the sham animals by the presence of Ca^{2+}. Calcium has been reported to prevent the breakdown of lysosomes (21,

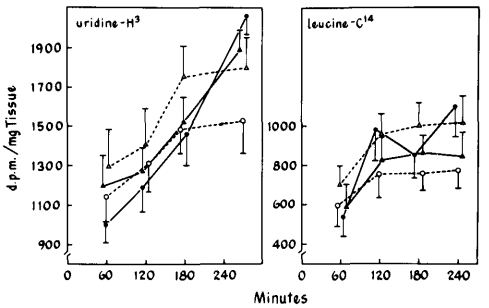

FIG. 7. The uptake of [^{14}C]leucine and [^{3}H]uridine into the intracellular fluid. The fluid was obtained from the TCA precipitation of the proteins of the 105,00*g* soluble supernatant solution. See legend to Fig. 2 for description of symbols.

22) which contain the enzyme involved in RNA degradation, RNase (23). Since Ca^{2+} levels are higher in the hypertrophied heart, the higher concentration of RNA in this tissue may be due to a direct inhibition of RNase, to an inhibition of its release from the lysosomes or decreased leakage out of the cell (21, 22). This may be of importance to the enhanced rate of protein synthesis since total RNA levels in the hypertrophied group remained higher for 2–3 weeks following aortic banding (1) whereas RNA synthesis was elevated only in the early stage of hypertrophy (1, 20).

Although tissue slice preparations have irregular, damaged cells, both RNA and protein are synthesized at a linear rate for 2–3 hr (7, 8, 19), suggesting that the cells are viable. In addition, no differences in the uptake of uridine and leucine were observed between the hypertrophy and sham groups (Fig. 7) using the tissue preparation. It would appear that the differences in the rate of RNA and protein metabolism are not due to a change in the rate of uptake of uridine or leucine into the sarcoplasmic pool from the extracellular media (8–10, 24–28). However, it is conceivable that the precursors may be transported more rapidly into an intracellular pool to be utilized faster in the hypertrophied heart, or that there may be small intracellular pools which have different intracellular concentrations of the precursors.

It can be observed that extracellular calcium enhances RNA and protein synthesis in both groups; but a more marked stimulation of the rate of sarcoplasmic protein synthesis by calcium was noted only in the sham group. The increased rate of basal protein synthesis in the hypertrophied group may be related to (i) increased levels of intracellular calcium or (ii) an increase in RNA concentration. In either case an increase in Ca^{2+} flux may be a significant determinant in the process associated with myocardial hypertrophy.

Summary. Myocardial protein synthesis was elevated 7 days after rats were subjected to experimental aortic outflow obstruction. Although RNA synthesis was not increased at this time, RNA concentration was elevated and may have provided for the observed increase in protein synthesis. A possible basis for the persistence of the high RNA levels was a decrease in the degradation of RNA. The increase in intracellular calcium observed in hypertrophied tissue may be involved in the maintenance of RNA concentration and in the increased rate of protein synthesis.

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