INDUCTIVE-LIKE STIMULATION OF RNA AND PROTEIN SYNTHESIS BY CALCIUM IN HEART SLICES

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SUMMARY: RNA and protein synthesis in the myocardium were stimulated after a short preincubation period with calcium. This elevation of macromolecular synthesis persisted in the absence of the ion for at least four hours. It appears that the uptake and/or the concentration of intracellular calcium induced a persistent and optimum enhancement of RNA and protein synthesis.

RNA and protein synthesis are increased in heart slices of animals exposed to β adrenergic stimulators [epinephrine, isoproterenol] (1,2), or to Ca^{2+} *in vitro* (3-6). Isoproterenol causes an increased deposition of Ca^{2+} inside the cell (7,8) and an increased rate of RNA (1) and protein synthesis (2). Although exogenous Ca^{2+} has been shown to enhance RNA (3,4) and protein synthesis (5,6) no correlation has been made between its increased uptake or concentration in the cell and degree of RNA and protein synthesis. In this report the rate of RNA and protein synthesis will be analyzed after a preliminary preincubation period with Ca^{2+} to determine if the formation of these macromolecules is stimulated and whether it remains elevated after removal of the cation.

METHODS

Sprague-Dawley rats, weighing 210-260 g, were fed water and food *ad libitum*. Myocardial tissue slices were prepared and incubated as described previously (3,5). In all these experiments the slices were pre and/or post incubated in media containing uridine 5,6 ^{3}H and leucine ^{14}C as described in the results. The preincubated slices were washed five times in a similar buffer at 37°C and then transferred to flasks containing fresh media.

After a pre and/or post incubation period for the designated time period described in figures and tables, the slices were processed as described for RNA (3) and protein (5). Since the proteins of the whole homogenate and the subcellular fractions showed the same response to calcium, only the proteins of the 105,000 x g soluble fraction are reported in this paper.

RESULTS

Effect of Ca^{2+} in pre-incubation media -- Slices were prepared in Ca^{2+} free media, preincubated 5, 10 or 20 minutes at 37°C in the presence and absence of 1.5 mM Ca^{2+}, and then transferred to flasks containing leucine ^{14}C and uridine 5,6 ^{3}H either in presence or absence of 1.5 mM calcium for a 60 minute post incubation period. As the length of preincubation time increased, a linear increase in the specific activity of protein and RNA was observed during the second incubation period even when calcium was omitted from the incubation fluid (Fig. 1). The rate of incorporation of both leucine and uridine, as shown in Fig. 1, increased twofold, when slices, preincubated in the absence of calcium, were incubated subsequently in its presence.

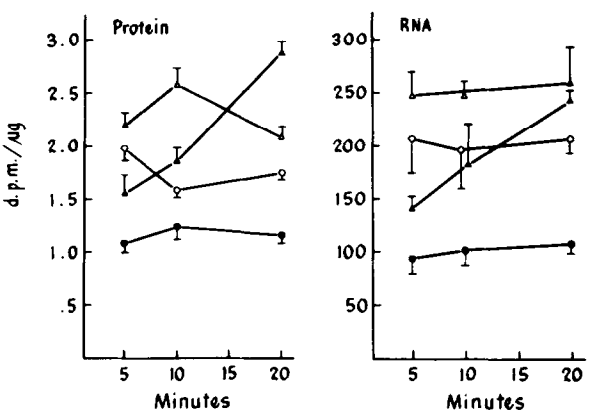
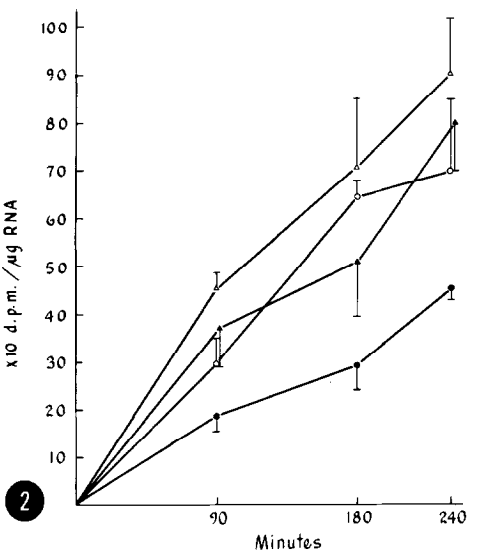


Figure 1. Effect of varied preincubation times with calcium on the rate of RNA and protein synthesis. Incubation conditions are described in text. Circles (○ , ●) preincubation in absence of Ca^{2+}; triangles (△ , ▲) preincubation in presence of 1.5 mM Ca^{2+}; open symbols (○ , △) post incubation in presence of calcium; closed symbols (● , ▲) post incubation in absence of 1.5 mM Ca^{2+}. Data are expressed as the average of 4-6 experiments.

Duration of calcium effect -- Slices were prepared in a calcium free media and then preincubated for 30 min in the buffer plus or minus calcium. During the post incubation period, the incorporation of leucine ^{14}C and uridine 5,6 ^{3}H into protein and RNA, respectively, proceeded at a faster rate when the slices had been preincubated in the presence of Ca^{2+} (Fig. 2 and 3).



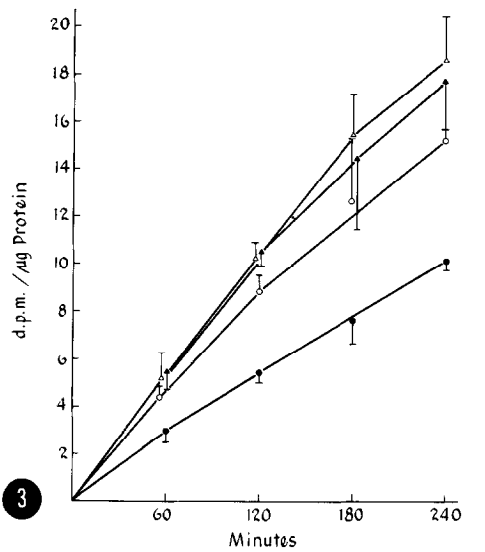


Figure 2. Duration of calcium stimulation on protein synthesis. Details of symbols are described in legend to Fig. 1.

Figure 3. Duration of calcium stimulation on RNA synthesis. Details of symbols are described in legend to Fig. 1.

This elevated rate of incorporation continued during the 4 hr post incubation period. The incorporation of labeled precursor was greatest when Ca^{2+} was present in both the preincubation and post incubation media. However, only an insignificant difference in the synthesis of RNA and protein was observed when slices, preincubated in the presence of calcium, were incubated in its absence (Fig. 2 and 3).

DISCUSSION

The synthesis of both RNA and protein in heart tissue was increased by a preliminary exposure to external Ca^{2+} (3-6). It is apparent that tissues prepared in a Ca^{2+} free media had a lower rate of formation of these macromolecules, suggesting that Ca^{2+} is an important ion involved in the augmentation of transcription and translation. It has been reported that Ca^{2+} aided in the stabilization of lysosomes (9), which contain enzymes involved in protein and RNA degradation (10). Our data, however, indicate that the mechanism of Ca^{2+} promotion of translation, at least in the myocardium, may not be through proteases, since the turnover of proteins was not altered after a four hour incubation (unpublished observation). The effect of Ca^{2+} on RNase is conflicting, since some investigators have noted an inhibition of RNase (11-14), whereas others (15-17) have observed no effect. In our experiments, a decrease in total RNA was observed after a three to four hour incubation in the absence of Ca^{2+} (unpublished observations). The Ca^{2+} inhibition of RNase is a possible mechanism, since total RNA levels were higher and diminished at a slower rate in the presence of Ca^{2+} (unpublished observations).

Exogenous Ca^{2+} appears to be required for the growth of various cells (18,19) and organs (20). The mechanism by which Ca^{2+} altered the growth rate of cells is not known but our investigations have indicated that intracellular Ca^{2+} may enhance the rate of both transcription and translation. This increased rate of macromolecular synthesis persisted for at least four hours. The duration of the Ca^{2+} effect on protein synthesis may depend on a mRNA with a long half life (21), a protein-bound-stable mRNA (22); it could be related to an increased rate of initiation of protein (23), or an increased movement of ribosomes along the RNA, or elongation (24).

It is of interest to observe that an increased rate of RNA (1) and protein synthesis (2) have been noted to occur in the myocardium after subcutaneous injection of β adrenergic stimulator, isoproterenol. In addition, this drug enhanced myocardial Ca^{2+} deposition. It is quite conceivable that the

enhanced rate of RNA and protein synthesis may be attributed to the increased levels of Ca^{2+} inside the cell. Our data support this concept since an exposure to exogenous Ca^{2+} lead to an elevated rate of synthesis of RNA and protein for at least four hours.

Mallov (2) has shown that the intracellular pool of amino acids was not increased even though protein synthesis was enhanced. Our investigations confirm this data since no difference in specific activity of leucine ^{14}C or uridine ^{3}H was observed regardless of the presence or absence of Ca^{2+} (3,5). It has been reported that the nucleoside triphosphate pool, the direct precursor to RNA, does not regulate the rate of RNA transcription (25). Cameron and LeJohn (26) have suggested that this ion may be coupling amino acid transport and protein synthesis in the water mold, Achyla. Hider et al. (27), Kostyo (28), Agostini and coworkers (29), and Van Venrooij et al. (30) suggested that precursor amino acids are located in the extracellular space, although it is conceivable that Ca^{2+} may augment the transport process across the cell membrane to site of protein and RNA synthesis. Our data indicate that the mechanism of Ca^{2+} action may be more complicated since an increased labeling of both RNA and protein was observed in slices which were preincubated in the presence of Ca^{2+}, followed by a second incubation in the presence of leucine ^{14}C and uridine 5,6 ^{3}H but without Ca^{2+}. It is conceivable that the membrane characteristics were changed such that more precursors were carried to sites of synthesis of RNA and protein.

Since Ca^{2+} can stimulate RNA and protein synthesis independent of each other (unpublished observations), there may be two separate mechanisms for the enhancement of RNA and protein synthesis. It is not known whether these two metabolic actions are a result of (1) direct Ca^{2+} effect on both RNA and protein synthetic pathways (31,32), (2) increased transport of precursors directly to site of RNA and protein synthesis (26,33), or (3) Ca^{2+} binding to or stimulating some factor(s) which induce RNA or protein synthesis. It can be noted that β adrenergic agents can affect both Ca^{2+} concentrations (8),

RNA (1) and protein synthesis (2) as well as cAMP (34). Since cAMP has been reported to be involved in enhancing protein synthesis (35-38), it is possible that the Ca^{2+} effect is mediated through this cyclic nucleotide. If the effects are dependent on the cyclic compound, then this compound must activate some process(es) which can persist for at least four hours. It is quite probable that Ca^{2+} itself can bind to an activator which regulates RNA and protein synthesis. To our knowledge the possibility of an inductive-like stimulation of either of these processes by the increased deposition or movement of Ca^{2+} inside the cell has not been previously reported.

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