Calcium Stimulation of Uridine-6-^{3}H Incorporation into RNA of Rat Myocardium (37051)

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Calcium has been reported to be involved in synthesis of proteins in different tissues (1–4). The mechanism by which calcium stimulated the formation of proteins is not understood; but it has been postulated that this divalent cation either augmented the transport of amino acids into the intracellular pool (4, 5) increasing the level of amino acids available for protein synthesis, or stimulated the synthesis of protein directly (6). In our investigations protein synthesis in rat ventricular slices was stimulated by calcium (1), but the intracellular radioactive amino acid pool was not altered. Previously calcium had been reported to be necessary for formation of specific RNA molecules of T-5 phage (7) and this investigation demonstrates that calcium also stimulated RNA synthesis in mammalian ventricular tissue.

Materials and Methods. The incubations were performed in 2.5 ml of a modified Krebs–Ringer phosphate (K-R) buffer (pH 7.4), containing 2 μCi [^{3}H]-uridine (sp act 25.4 Ci/mmole, New England Nuclear Corp.) and all the amino acids (Sigma Co.) at their normal serum concentrations (8). Calcium at final concentrations ranging from 0.01 to 4.0 m*M* was added to the buffer prior to placing the ventricular slices (140 mg) into the flasks. In the RNA inhibition experiments, 10 μg/ml actinomycin D (Merck and Co.) were added to the flasks. After gassing for 1 min with 95%–5% (O_2–CO_2) the slices were incubated for either 90 min or the varied time periods as shown in Fig. 1b on a gyrotory shaking bath at 37°. The tissue was rinsed 3 times with 0.25 *M* sucrose in the K-R buffer and homogenized in the same buffer. The homogenate was centrifuged at 600*g* for 10 min, and the pellet was washed 3 times with the homogenizing media.

The supernatant solution was centrifuged successively at 8500*g* for 10 min, 13,000*g* for 20 min and finally at 105,000*g* for 45 min. The pellets were discarded and the final supernatant solution was assayed for radioactivity after removal of proteins and RNA by precipitation with 20% (w/v) trichloroacetic acid (TCA). RNA was extracted from the 600*g* fraction by the method of Munro and Fleck (9). The washings and original TCA-soluble supernatant solution were pooled and a 300 μl aliquot removed for counting in 10 ml of scintillation fluid [2:1 toluene: methyl Cellosolve containing 4 g PPO and 100 mg POPOP/liter]. RNA was analyzed by the method of Ceriotti (10) using yeast RNA as a standard. The samples were counted in a Packard liquid scintillation counter at 20% efficiency for tritium.

Results and Discussion. Calcium at concentrations ranging from 0.01 to 4 m*M* stimulated the uptake of [^{3}H]-uridine into RNA (Fig. 1a). It appears that there was an initial stimulation at low concentrations of calcium (0.01–0.10 m*M*) attaining a value 2.5 times the zero calcium level. As the concentration of calcium was elevated, the incorporation of uridine continued to increase steadily with a peak at the physiological concentration, 2.5 m*M* calcium, with no significant change at 4.0 m*M* (Fig. 1a).

The incorporation of [^{3}H]-uridine into RNA was linear for the first 60 to 120 min at all the concentrations studied (Fig. 1b). However, at the high concentration of calcium (2.0 and 2.5 m*M*) the rate of incorporation began to plateau after 120 min. By the end of 180 min the absolute amount of incorporation was similar at all the higher cal-

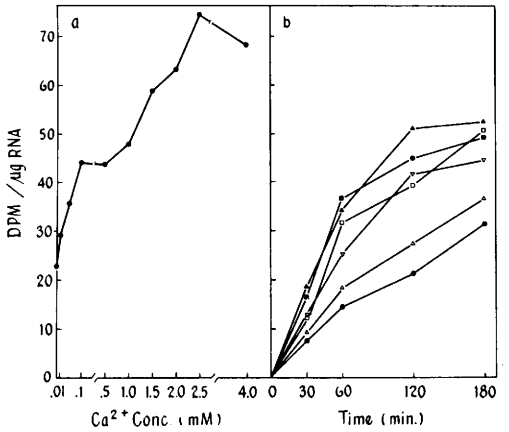

FIG. 1a. Calcium stimulation of the incorporation of [^{3}H]-uridine into RNA. The slices were incubated 90 min in a shaking water bath. RNA was extracted with 0.3 *N* KOH for 1 hr from the 600*g* pellet which had been precipitated with 20% TCA as described in Methods. The DNA and proteins were removed by precipitation with 1.0 *N* PCA. The data are expressed as dpm/μg RNA and the result of 2 experiments. (b) Time profiles for the effect of calcium on the stimulation of RNA synthesis. RNA was extracted as described in Methods. The data are expressed as dpm/μg RNA and the average of 2 experiments. (●—) 0 Ca^{2+}; (△—) 0.5 m*M* Ca^{2+}; (▽—) 1.0 m*M* Ca^{2+}; (□—) 1.5 m*M* Ca^{2+}; (■—) 2.0 m*M* Ca^{2+}; (▲—) 2.5 m*M* Ca^{2+}.

cium concentrations.

Since calcium had been suggested to stimulate the transport of amino acids into the intracellular pool, the level of [^{3}H]-uridine in the TCA-soluble fraction of sarcoplasm may be altered by the various calcium concentrations. No significant differences in the amount of [^{3}H]-uridine in the sarcoplasm was noted irrespective of the calcium concentration (Table I). The mechanism of increased RNA synthesis does not appear to involve an increased pool size in the cell; but the data does not rule out the transport of uridine directly to the RNA biosynthetic site in the nuclei. In order to confirm the calcium stimulation of RNA synthesis, actinomycin D was added to the flasks. Ten micrograms per milliliter of Act D was sufficient to inhibit incorporation of uridine-6-^{3}H into RNA by 50% and completely reversed the calcium stimulation (Table II).

TABLE I. Uptake of [^{3}H]-Uridine into the TCA-Soluble Fraction of the Sarcoplasm of Ventricular Slices.[a]

Ca^{2+} (m*M*)	dpm/mg slice
0	403 ± 64
0.01	467 ± 114
0.05	403 ± 40
0.10	513 ± 80
0.50	612 ± 95
1.0	437 ± 72
1.5	418 ± 20
2.0	415 ± 58
2.5	506 ± 107
4.0	389 ± 103

[a] The sarcoplasm was obtained by centrifuging a 20,000 *g* supernatant at 105,000 *g* for 45 min to remove the microsomes. The sarcoplasmic proteins and RNA were removed by precipitation with 20% TCA. A 100 μl aliquot was used for counting and the data are expressed as dpm/mg slice ± SEM. The data are the average of 4 observations.

The mechanism of increased RNA synthesis by calcium may be mediated via a membrane factor or directly by the divalent cation. Exogenous calcium may alter intracellular calcium causing a stimulation of RNA synthesis. The data definitely indicates that calcium is necessary for optimal RNA biosynthesis in rat ventricular slices. This increased RNA biosynthesis by calcium may be a factor in the observed protein stimulation by the cation.

Summary. Calcium stimulated RNA synthesis in rat heart ventricular slices. Concentrations of calcium ranging from 0.01 to 4.0 m*M* augmented the incorporation of [^{3}H]-uridine into RNA, in 2 phases, one occurring at low concentrations of calcium (0.01–0.1 m*M*) and the other around the physiological concentration (2.5 m*M*). The rate of incorporation is linear for 60–90 min at all concentrations but plateaued after 120

TABLE II. Effect of Actinomycin D on Calcium Stimulation of RNA Synthesis.

Additions	RNA[a] (dpm/μg)	
	0 Ca^{2+}	1.5 mM Ca^{2+}
None	25.2 ± 3.7	40.4 ± 5.4
Act D, 10 μg/ml	13.6 ± 1.9	13.8 ± 1.4

[a] Mean ± SD of four observations.

min at the high concentions. Actinomycin D completely inhibited the calcium effect. Calcium appeared to be required for maximal RNA biosynthesis. This increased rate of synthesis of RNA may be a factor involved in the increased stimulation of protein anabolism by calcium.

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