Effect of calcium and calmodulin on RNA synthesis in isolated nuclei from rat liver cells

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1. INTRODUCTION

Calcium regulates a great variety of intracellular processes [1–4]. In muscular contraction [5] or the activation of adenylate [6,7], calcium, acting as a coupling or second messenger [4], binds to specific proteins like troponin [5] or calmodulin [6–8] to exert its action.

In RNA synthesis, activation by calcium is not well understood. Calcium stimulates RNA synthesis in slices of ventricular tissue [9], GH_3 cells [10] and cultures of chicken pectoralis muscle [11]. But only indirect evidence is available about the molecular mechanism of such activation. Calcium-dependent protein kinases in the nuclei of rat liver cells [12], the participation of calcium in the phosphorylation of non-histone proteins [13,14] and the presence of calmodulin in the nuclei of some cells [15], suggests that calcium could stimulate RNA synthesis through interaction of calcium with calmodulin, activation of calcium-dependent protein kinases by this calcium–calmodulin complex, phosphorylation of specific non-histone proteins and, finally, derepession of the genome.

To test this hypothesis, we have investigated the effects of calcium, calmodulin and the calmodulin inhibitor, chlorpromazine, on RNA synthesis in isolated nuclei from rat liver cells. The results on the stimulation of RNA synthesis by calcium were not confirmed by our study, which has shown instead that calcium inhibits RNA synthesis and that this effect (again unlike the previous hypothesis) is not dependent on calmodulin.

2. MATERIALS AND METHODS

[2,6-^{3}H]UTP was obtained from New England Nuclear. ATP, GTP, UTP, CTP, albumin, calmodulin and chlorpromazine were from Sigma. All other reagents were of analytical grade.

2.1. *Isolation of nuclei*

The livers were obtained from male Wistar albino rats (170–200 g body wt). Nuclei were isolated by differential centrifugation according to [16].

2.2. *Determination of RNA synthesis*

This was done as in [17]: The assay was initiated with the addition of nuclei to the reaction mixture. The final mixture contained 35–55 μg nuclear protein, 12.5% glycerol, 150 mM KCl, 5 mM $MgCl_2$, 25 mM Tris–HCl (pH 8), 2.5 mM mercaptoethanol, 0.4 mM ATP, GTP, CTP, and 0.05 mM [^{3}H]UTP (0.65 Ci/mmol) in 0.1 ml. The mixture was incubated for 10 min at 37°C. The reaction was stopped by addition of 5 ml 10% trichloroacetic acid/40 mM sodium pyrophosphate. The precipitated material was collected by filtration through Whatman GF/A papers, and washed with 50 ml of the same solution and 5 ml ethanol. Radioactivity was determined in a liquid scintillation spectrophotometer. Protein was determined by the biuret method [18].

3. RESULTS

3.1. *Effect of calcium and calmodulin on RNA synthesis*

To study the effect of calcium on the kinetics of RNA synthesis, the nuclei were incubated in the

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Table 1

The effect of Ca^{2+} and calmodulin on RNA synthesis in isolated nuclei from rat liver cells

Incubation conditions	RNA synthesis (% activity)
1. EGTA	100
2. Ca^{2+}	62.9
3. Ca^{2+} + calmodulin	61.2
4. Ca^{2+} + calmodulin + chlorpromazine	63.1

Nuclei were incubated for 20 min in the presence of: (1) 1.25 mM EGTA; (2) 0.125 mM $CaCl_2$; (3) 0.125 mM $CaCl_2$ + 1 μM calmodulin; (4) 0.125 mM $CaCl_2$ + 1 μM calmodulin + 0.25 mM chlorpromazine. The synthesis of RNA was assayed as in section 2; results are the means of 3 separate experiments

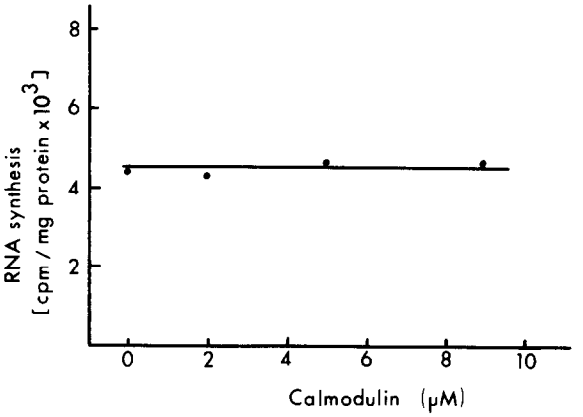

Fig.2. Effect of calmodulin on RNA synthesis. Nuclei were incubated for 20 min. in presence of 5 μM $CaCl_2$ and different concentrations of calmodulin. The synthesis of RNA was assayed as described. Results are the means of 2 separate experiments

presence of 1.25 mM EGTA or 0.125 mM $CaCl_2$ for variable times. The initial rate of the RNA synthesis was lower in the presence of calcium than in the presence of EGTA (fig.1). Incubations for > 20 min were not studied because the [^{3}H]UTP incorporation reaction is not involved in the initiation of RNA synthesis but only during chain elongation [17]. Calcium also diminished the maximal incorporation of [^{3}H]UTP (see fig.1 and table 1), suggesting a decrease in the total number of active initiation sites. Since calcium participates in a variety of processes through its complexing to calmodulin [1–3,8], we have also studied the influence of this protein on the inhibition of RNA synthesis mediated by calcium. Two types of experiments were done: in one, calmodulin was present in the incubation mixture; in the other, we used chlorpromazine, a specific inhibitor of calmodulin-dependent processes [19]. Inhibition by calcium was not influenced by the presence of calmodulin or chlorpromazine (table 1). Calmodulin varied from 0–10 μM without any detectable effect on the inhibition mediated by calcium (fig.2).

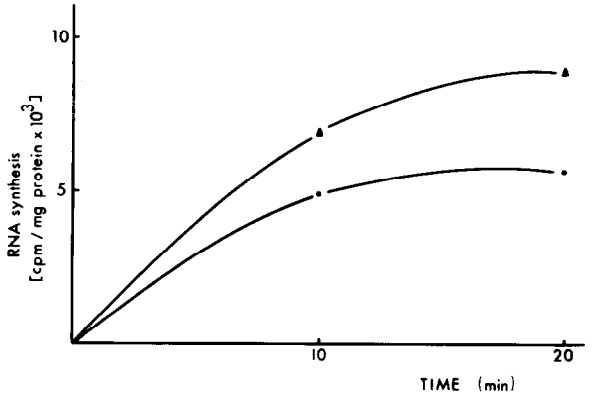

Fig.1. Effect of calcium on RNA synthesis. Nuclei were incubated in presence of 1.25 mM EGTA (▲) or 0.125 mM $CaCl_2$ (●) for different times. The synthesis of RNA was assayed as in section 2. The zero-time value obtained was subtracted from other values. Results are the means of 3 separate experiments

3.2. *Concentration dependence of the calcium-mediated inhibition*

RNA synthesis was studied by incubating nuclei in the presence of different calcium concentrations. Calcium inhibited RNA synthesis in two ranges of concentration, between 0–10 μM, and > 0.5 mM (fig.3).

4. DISCUSSION

The role of calcium in processes such as muscular contraction, hormone secretion and neurotransmitter release is well documented [1–5,8], whilst participation of this cation in the RNA synthesis remains obscure. Here we have demonstrated an inhibitory

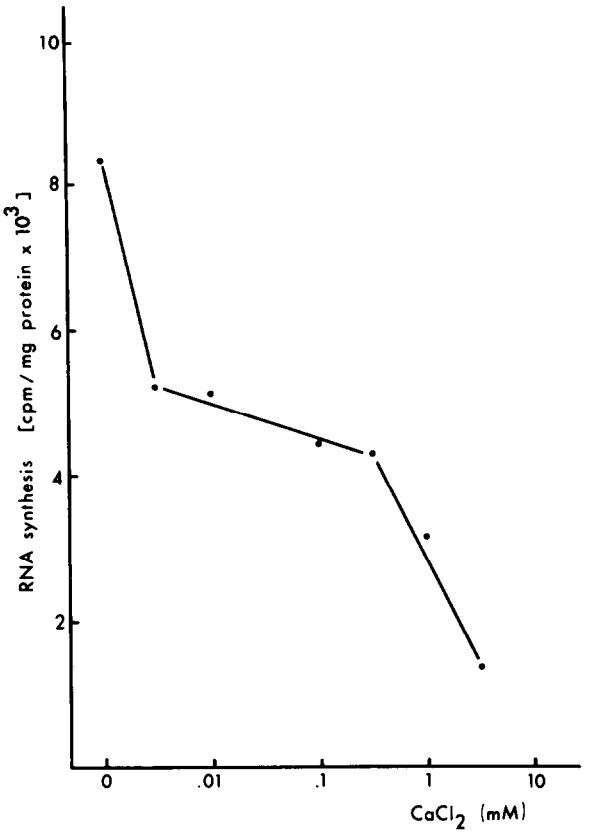

Fig.3. Concentration dependence of calcium inhibition of RNA synthesis. Nuclei were incubated for 20 min in presence of different concentrations of calcium. Synthesis of RNA was assayed as described. The maximum value of RNA synthesis was obtained by incubating the nuclei in presence of 1.25 mM EGTA. Results are the means of 3 separate experiments

action of calcium on RNA synthesis in disagreement with [9–11]. We have used isolated nuclei instead of slices [9] or cell cultures [10,11]. The differences in the experimental systems could explain, in part, the disagreement. The activating action of calcium in slices or cell cultures could be a more complex phenomenon than the simple interaction of the ion with some nuclear proteins [11,20].

The calcium concentration in the cytosol and probably in the nucleosol, is 1 μM [21]. A first range of inhibition appears using calcium concentrations within the same order of magnitude (fig.3). These data suggest that the inhibitory action of calcium on RNA synthesis can be significant in vivo. Therefore its physiological significance remains to be studied.

Since calcium activates a variety of processes by complex formation with calmodulin [1–3,8], we have also studied the influence of the protein and the specific inhibitor of calmodulin-dependent processes, the antipsychotic drug chlorpromazine [19]. The results obtained show that the effect of calcium was independent of calmodulin.

With respect to the inhibition of RNA synthesis produced by high calcium concentrations, it has been reported that hepatic damage produced by phallidin is associated with an increase in the cytoplasmic concentration of calcium [22]. Therefore, it is possible that inhibition of RNA synthesis at high calcium concentrations could be one of the events involved in the process of hepatic damage by toxic agents.

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