EFFECT OF Ca^{++} IONS ON THE RATE OF RNA AND PROTEIN SYNTHESIS IN CELL-FREE SYSTEMS OF HEART AND LIVER

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Ca^{++} ions are involved in the regulation of muscle contractility, play an important role in proliferation processes [1], and participate in the control of hormone secretion [2] and activation of phosphorylase systems and proteolysis [3]. Meanwhile the relationship of Ca^{++} to RNA and protein synthesis has not yet been determined and data in the literature are contradictory. According to some workers [4], for instance, Ca^{++} does not affect rRNA synthesis in mammalian cell cultures, whereas according to others [5], Ca^{++} stimulates the synthesis of polyuridylic acid in a cell-free system. However, autoradiographic studies have shown that the rate of RNA synthesis falls sharply in neurons in response to loading with Ca^{++} [6].

It has also been reported that Ca^{++} increases the rate of protein synthesis in tissue slices [7] and in mammalian cell cultures [8], although the molecular mechanism of this effect is not clear.

The aim of this investigation was to study the effect of Ca^{++} ions on the rate of RNA and protein synthesis. The investigation was conducted on cell-free systems of RNA and protein synthesis of the heart and liver.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. Liver and heart nuclei were isolated by the sucrose method [9]. To determine RNA polymerase activity of the nuclei an incubation medium (0.5 ml) of the following composition (in μM) was used: for RNA of polymerase I (system I) — Tris-HCL (pH 7.9) 50, KCl 25, $MgCl_2$ 6, $(NH_4)_2SO_4$ 50, GTP and CTP 0.6 of each, UTP 0.06, creatine phosphate 5, creatine phosphokinase, and 2 μCi of ^{14}C-UMP. To determine RNA polymerase II activity the same incubation mixture was used except that the $MgCl_2$ was replaced by 2 μM $MnCl_2$ and the $(NH_4)_2SO_4$ concentration was 160 μM. The reaction was triggered by the addition of nuclear suspension (50 μg DNA). To inhibit RNA polymerase II activity, α-amanitine was added to system I in a dose of 1 μg/ml. The cytosol fraction, isolated by the method in [9] with certain modifications, was added at the rate of 8-10 mg protein to 1 ml. Ca^{++} ions ($CaCl_2$) were added over a wide range of doses, including physiological. EGTA was added to the system in concentrations of 1.2×10^{-6} and 1.5×10^{-6} M. The samples were incubated at 36°C for 15 min. A 10% solution of TCA containing 0.04 M sodium pyrophosphate was then added, and the residue was washed on Millipore filters of the Aufs type. Changes in RNA synthesis were judged from incorporation of radioactive uridine monophosphate (^{14}C-UMP) into the acid-insoluble fractions of the nuclei. The cell-free system of protein synthesis was reconstituted from ribosomes and cell sap, passed through Sephadex G-25 (coarse). The incubation medium (1 ml) contained 100-125 μg ribosomal RNA (rRNA), cell sap (0.8-1 mg protein), 5 mM ATP, 0.6 mM UTP, 10 mM creatine phosphate, 50 μg creatine phosphokinase, 2 mM EDTA, 1 mM dithiothreitol, 125 mM KCl, 30 mM Tris-HCl (pH 7.6), 7.5 mM $MgCl_2$, a mixture of unlabeled amino acids with 0.04 mM of each, and 1 μCi of ^{14}C-leucine and ^{14}C-lysine. The samples were incubated for 45 min at 37°C and washed with TCA solution on Aufs Millipore filters [10]. Radioactivity of the samples was determined on a scintillation counter in toluene solvent containing PPO and POPOP, and expressed in cpm per sample.

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TABLE 1. Effect of Ca^{++} on RNA Synthesis in Isolated Heart and Liver Nuclei

Conditions of incubation	Incorporation of ^{14}C-UMP, cpm per sample			
	heart nuclei		liver nuclei	
	rRNA	mRNA	rRNA	mRNA
Control	300	650	1200	2600
Addition of Ca^{++} (in M):				
$5 \cdot 10^{-8}$	240	400	1000	1600
10^{-6}	180	260	800	1100
10^{-4}	120	130	500	600
Control + EGTA ($1.2 \cdot 10^{-6}$ M)	330	600	1100	2500
$+Ca^{2+}$ ($5 \cdot 10^{-8}$M) + EGTA ($1{,}5 \cdot 10^{-6}$ M)	350	620	1000	2400
$+Ca^{2+}$ (10^{-6} M) + EGTA ($1{,}5 \cdot 10^{-6}$ M)	320	600	1100	2400
Control + cytosol	450	900	2200	3200
Control + cytosol + Ca^{2} (10^{-6} M)	250	420	900	1200

EXPERIMENTAL RESULTS

The effect of Ca^{++} in a cell-free system of RNA synthesis containing heart and liver nuclei was investigated in the experiments of series I. Under these conditions activity of RNA polymerase I, the enzyme responsible for rRNA synthesis, and also of RNA polymerase II, an enzyme involved in the synthesis of messenger RNA (mRNA) was determined. The results of these experiments showed (Table 1) that exogenous Ca^{++}, added to heart and liver nuclei in a concentration of 5×10^{-8} M, depresses rRNA synthesis by 20% and mRNA synthesis by 40%. A higher concentration of Ca^{++} (10^{-6} M) inhibits rRNA synthesis by 40% and mRNA synthesis by 50%.

It is generally considered that the Ca^{++} concentration in the cytoplasm of the resting myocyte is 10^{-8} M.

During excitation of the muscle cell it rises to 10^{-6} M [11]. The Ca^{++} concentration in the hepatocyte cytoplasm is 10^{-7} M [12]. Consequently, addition of Ca^{++} in a dose of 10^{-8} M corresponds to a physiological concentration, whereas a dose of 10^{-6} M, allowing for the presence of endogenous Ca^{++}, is rather above the physiological level.

With high concentrations of Ca^{++} (10^{-4} M) the rate of transcription in heart and liver nuclei fell sharply: rRNA synthesis was reduced by 60% and mRNA synthesis by 80%. Thus Ca^{++} ions cause dose-dependent inhibition of transcription in heart and liver nuclei, and synthesis of mRNA is inhibited particularly sharply.

To obtain proof of the inhibitory effect of Ca^{++} on RNA synthesis, we added EGTA to the cell-free system. This compound forms chelated complexes selectively with Ca^{++} and thereby blocks its action. EGTA was added in a concentration of 1.5×10^{-6} M to incubation medium containing and not containing exogenous Ca^{++}. It will be clear from Table 1 that the addition of EGTA to the cell-free system completely restored the rate of transcription. The rate of RNA synthesis was unchanged in a system not containing exogenous Ca^{++}.

The writer showed previously [9] that the cytoplasmic fraction of heart and liver (the S-100 fraction) contains organ-nonspecific protein factors sharply increasing the rate of rRNA and mRNA synthesis. It was accordingly interesting to add the S-100 cytoplasmic fraction to a cell-free system of RNA synthesis containing exogenous Ca^{++}. Table 1 shows that in the presence of added Ca^{++} (10^{-6} M) the S-100 cytoplasmic fraction had no stimulating action on the rate of transcription in isolated heart and liver nuclei.

In the next series of experiments the effect of Ca^{++} ions on the rate of translation was studied. The cell-free system of protein synthesis was reconstituted from polysomes and cell sap from heart muscle [10]. Ca^{++} was added to the incubation medium in doses from 10^{-7} to 2.5×10^{-6} M. The results showed (Table 2) that in a cell-free system of protein synthesis Ca^{++} ions do not affect the rate of translation.

Thus Ca^{++} inhibits the rate of transcription in isolated heart and liver nuclei, and inhibits mRNA synthesis particularly sharply. Ca^{++} does not affect the rate of translation.

An increase in the rate of protein synthesis under the influence of added Ca^{++} has been described for tissue slices [7] and in mammalian cell culture [8]. These data do not agree

TABLE 2. Effect of Ca^{++} on Incorporation of Labeled Amino Acids into Heart and Liver Ribosomes

Experimental conditions	Radioactivity, in cpm per sample	
	heart	liver
Control	4 000	10 000
Addition of Ca^{++} (in M):		
10^{-8}	3 900	11 000
10^{-7}	3 800	10 500
10^{-6}	4 100	11 000
$2{,}5 \cdot 10^{-6}$	3 700	11 000

with the results of the present experiments. It may be that the effect of Ca^{++} in the experiments cited was indirect and that other components of metabolic pathways were involved. Under the conditions of a cell-free system of protein synthesis, the rate of translation was unchanged after addition of exogenous Ca^{++}.

As already stated, data in the literature on the effect of Ca^{++} on the rate of RNA synthesis are extremely contradictory. In the present experiments, using a cell-free system containing heart and liver nuclei, Ca^{++} ions even in physiological doses inhibit the rate of transcription considerably. Synthesis of mRNA was depressed particularly sharply. When these data are discussed it must be recalled that the rate of RNA synthesis is largely determined by protein factors for initiation and elongation of transcription. An increase in the Ca^{++} concentration may lead to activation of phosphorylation of these factors and, consequently, to their inhibition. A similar situation is known for translation, when reversible phosphorylation of the initiation factor eIF-2 is the stage limiting the rate of protein synthesis [13]. Inactivation, dependent on Ca^{++}, of phosphorylation has also been described for certain enzymes [14]. Meanwhile an increase in the Ca^{++} concentration causes activation of Ca^{++}-dependent proteinases [3]. Both these processes may lead to inactivation of protein transcription factors and thus to a decrease in the rate of RNA synthesis. In this respect it is an interesting fact that addition of the S-100 cytoplasmic fraction to an incubation medium containing exogenous Ca^{++} does not have its characteristic stimulating action on the rate of transcription. It must also be recalled that the Ca^{++} concentration in the nucleoplasm may be considerably lower than in the cytoplasm. Accordingly, fluctuations in the Ca^{++} concentration that are too small to affect the metabolism of the cytoplasm may prove significant for processes taking place in the nucleus. It can be postulated that a change in the Ca^{++} concentration in the nucleoplasm, determined by its level in the cytoplasm, plays an important role in the mechanism of regulation of transcription.

High Ca^{++} concentrations have been found in the cytoplasm of the heart and skeletal muscles in cardiomyopathies [15] and in certain dystrophies, notably Duchenne's dystrophy [16]. The rate of RNA and protein synthesis is reduced under these circumstances, but the rate of proteolysis is increased. Accumulation of Ca^{++} in the cytoplasm of muscle tissue cells during dystrophy and myopathies may be the cause of the decrease in the rate of macromolecular synthesis. This may be of significant importance for the elucidation of the pathogenesis of these diseases.

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ROLE OF CATECHOLAMINES IN THE MECHANISM OF HEART DAMAGE IN RATS WITH ALCOHOL WITHDRAWAL SYNDROME

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It is generally accepted that the development of alcohol cardiomyopathy is due primarily to the toxic action of ethanol. Meanwhile experiments on rats have shown that marked disturbances of the contractile function and carbohydrate metabolism of the heart arise, not at the height of alcohol intoxication, but after the action of ethanol has ended, namely in the period of development of the withdrawal syndrome [7]. Because of activation of the sympathicoadrenal system observed in the alcohol withdrawal syndrome [3, 11], catecholamines (CA) can be regarded as one possible pathogenetic factor of the disturbance of cardiac activity.

The aim of this investigation was to study the rate of disappearance of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) from the perfused heart and the histochemical determination of CA in the rat heart at various times after intensive alcoholization, leading to the development of a withdrawal syndrome.

EXPERIMENTAL METHOD

Experiments were carried out on 82 noninbred male albino rats aged 2-3 months. The alcohol withdrawal syndrome was produced by injecting a 25% solution of ethanol in a dose of 4-5 g/kg body weight into the stomach at intervals of 12 h for 5 days [1]. The animals were killed by decapitation 3-6 h and 1, 2, 3, and 7 days after the last injection of ethanol.

TABLE 1. Rate of Disappearance of Enzymes from the Perfused Rat Heart at Different Times after Alcohol Withdrawal

Time after last injection of ethanol (n =7)	CPK	LDH
Control	0,732±0,053	0,609±0,085
2—6 h	1,086±0,192	0,546±0,085
1 day	1,212±0,205*	0,685±0,109
2 days	1,321±0,154*	0,820±0,103
3 days	1,809±0,081*	1,028±0,068*
7 days	1,728±0,153*	0,797±0,121

Legend. Here and in Table 2: *$p < 0.05$ compared with control. n) Number of animals in each period of observation.

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