IN VITRO PROTEIN SYNTHESIZING ACTIVITY OF RAT LIVER AS INFLUENCED BY A PHYSIOLOGICAL DOSE OF CORTISONE AND DIBUTYRYL CYCLIC AMP

Jiro Hoshino, Uta Kühne, Gabriele Studinger and Hans Kröger
Robert Koch-Institut, 1000 Berlin 65, Germany-West

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SUMMARY

A single administration of a low dose of cortisone acetate (0.2 mg/100 g) into adrenalectomized rats reduces the cell-free protein synthesizing activity of isolated total liver polysomes by 60 % after 90 min. DBcAMP also inhibits markedly the protein synthesizing activity but its effect is due to an inactivation of soluble fraction. Combined administration of both drugs yields polysomes active more than twice than those of untreated control. Inhibition by the glucocorticoid was restricted only to the membrane-bound polysomes.

INTRODUCTION

Glucocorticoids and DBcAMP (or glucagon) have been shown to specifically stimulate the synthesis of several enzymes in rat liver and in cultured hepatoma cells (1,2). Kim and Kim (3) recently demonstrated that a single injection of pharmacological amount of glucocorticoid causes a rapid transitory inhibition of the total liver protein synthesis, which is followed by an increased rate of protein synthesis. Available data concerning the effect of cyclic AMP in vivo on the rate of total protein synthesis show a considerable inhibition by the cyclic nucleotide. Recent work of Klaipongpan et al. (4) demonstrated the requirement of cyclic AMP for a membrane component to manifest its inhibitory action in the cell-free protein synthesizing system. We showed previously that a non-inducing physiological amount of glucocorticoids enhances the DBcAMP-mediated induc-

ABBREVIATIONS: DBcAMP = $N^6,O^{2'}$-dibutyryladenosine 3',5'-monophosphate; TAT = L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5).

tion of tyrosine aminotransferase (TAT) in rat liver (5) and in cultured rat liver cell line (6). In this communication we present changes in the in vitro protein synthesizing activity under the influences of a physiological dose of cortisone and of DBcAMP. A striking enhancement in the activity was observed, if the both drugs are administered together.

MATERIALS AND METHODS

Female Wistar rats weighing 130-150 g were bilaterally adrenalectomized and used for experiments 7-10 days after operation. Cortisone acetate (0.2 mg/100 g body weight, Ciba AG, Wehr/Baden) and DBcAMP (3 mg/100 g, Boehringer Mannheim GmbH, Mannheim) were intraperitoneally injected into the rats and they were killed 90 min later. Livers from 2-3 rats were pooled and homogenized in 2 vol. of TKM buffer (50 mM Tris-HCl, pH 7.6, 25 mM KCl and 5 mM $MgCl_2$) containing 1 mM dithiothreitol (DTT) and 0.125 M sucrose in a Potter-type homogenizer. Post-mitochondrial supernatants were prepared by centrifuging homogenates at 10.000 xg for 10 min and an aliquot was further centrifuged at 165.000 xg for 90 min to prepare the soluble fraction (S-165). Another aliquot was subjected to the preparation of total-, free- and bound (released from membrane) polysomes according to Blobel and Potter (7). The assay mixture for cell-free protein synthesis contained, in a final volume of 1 ml, the following: 35 mM Tris-HCl (pH 7.5), 1 mM ATP, 0.25 mM GTP, 7.4 mM creatine-phosphate, 10 µg creatine kinase, 75 mM NH_4Cl, 4 mM Mg-acetate, 1 mM DTT, 0.05 mM 19 amino acids and 0.3 µCi of L-[U-^{14}C] leucine (330 mCi/mmol). Polysomes and S-165 were exactly adjusted to the final concentrations of 5.0 A 260 units and 3.2 mg protein, respectively. The mixtures were incubated at 21° for 10 min and the reaction was terminated by the addition of 2 ml of 10 % TCA containing 2 % Celite. The hot TCA-insoluble radioactivity was counted as described by Sarma et al. (8). Data are the means of 3 determinations.

RESULTS AND DISCUSSION

The cell-free systems shown in Exp. I in Table 1 are constituted from total polysomes and soluble fractions of the livers obtained from untreated control- and cortisone-treated rats. It is evident that a single injection of cortisone leads to a 50 % inhibition of cell-free protein synthesis after 90 min. Furthermore the inhibitory effect is entirely due to a reduced ability of polysomes to incorporate the precursor and does not depend on the soluble fraction. The results of Exp. IV in Table 1 demonstrate that continous presence of a physiological amount of

TABLE 1 [^{14}C]leucine incorporation into proteins in cell-free systems constituted from total liver polysomes and S-165 from differently treated rats.

Fraction		[^{14}C]leucine Incorporation	
Polysome	Soluble	cpm/tube	per cent of control
I			
Control	Control	1904	100
Control	Cortisone	1946	102
Cortisone	Control	797	42
Cortisone	Cortisone	974	51
II			
Control	DBcAMP	1133	60
DBcAMP	Control	1710	90
DBcAMP	DBcAMP	1019	54
III			
Control	Cortisone + DBcAMP	1534	81
Corrtisone + DBcAMP	Control	4498	236
Cortisone + DBcAMP	Cortisone + DBcAMP	3500	184
IV			
Intact	Intact	854	47
Adrenal-ectomized	Adrenal-ectomized	1833	100

Soluble fractions (S-165) and total polysomes were prepared from adrenalectomized rats 90 min after i.p. injection of either cortisone acetate (I), DBcAMP (II) or cortisone + DBcAMP (III). Those of control rats were from untreated adrenalectomized rats. For exp. IV intact and adrenalectomized rats of the same age were used.

glucocorticoids in vivo actually inhibits protein synthesis in vitro, since the livers of intact rats are able to synthsize proteins at a rate of only 47 % of that of adrenalectomized rats.

As previously shown the change in the plasma glucocorticoid level within a physiological range does not affect the basal TAT level in rat liver, although a dramatic change in the response of TAT synthesis to DBcAMP (5) or in the template activity of chromatin (9) occurs. Recently Kim and Kim (3) observed an initial transitory inhibition of total protein synthesis in the livers of intact rats caused by a pharmacologic dose of glucocorticoids, whereas a consistent inhibition occured by multiple administration. In marked contrast, polysomal fraction is not responsible for the inhibition (46 %) of protein synthesis caused by the administration of DBcAMP (Exp. II in Table 1). The activity of the soluble fraction to promote protein synthesis is remarkably reduced. Several studies have implicated the inhibitory effect of cyclic AMP on the total protein synthesis in rat liver (4, 10), presumably through the cyclic AMP-dependent phosphorylation of ribosomal proteins (11), although no significant change in the ribosomal function was seen by Eil and Wool (12) in the reconstituted phosphorylated ribosome system. Our present result argues against the inhibition through the phosphorylation of ribosomal proteins. Some modification in the soluble factor may be responsible for the inhibition of protein synthesis caused by DBcAMP, although a marked disaggregation of heavy polysomes was observed 90 min after injection of the cyclic AMP (data are not shown), as opposed to the results of Ayuso-Parrilla and Parrilla (13), who found no disaggregation of polysomes after glucagon administration. Exp. III in Table 1 shows that combined administration of cortisone and DBcAMP yields polysomes which are more than twice as active as the control polysomes, whereas it slightly inactivates the soluble fraction. Fig. 1 illustrates the time course

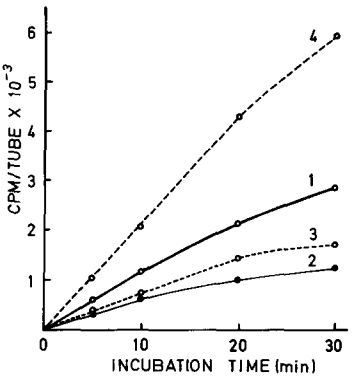

Fig. 1 Time course of [^{14}C]leucine incorporation into proteins in cell-free systems consist of polysomes and S-165 fractions from differently treated rats. Adrenalectomized rats were i.p. injected with the drugs and polysomes and S-165 were prepared as described under Methods. 1. control polysome-control S-165, 2. cortisone polysome-control S-165, 3. control polysome-DBcAMP S-165, 4. (cortisone + DBcAMP) polysome-control S-165.

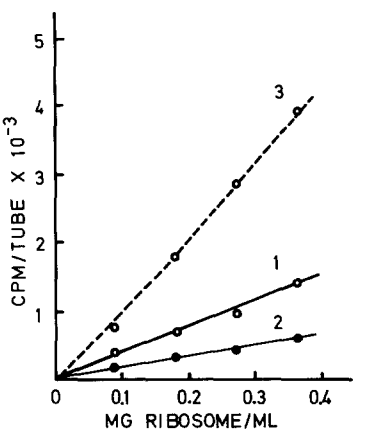

Fig. 2 Dependence on the polysome concentration of [^{14}C]-leucine incorporation into proteins in cell-free system. Adrenalectomized rats were treated as in Fig. 1. 1. control polysome-control S-165, 2. cortisone polysome-control S-165, 3. (cortisone + DBcAMP) polysome-control S-165.

of incorporation of [^{14}C]leucine into proteins in the cell-free systems reconstituted from polysomes and S-165 fractions of differently treated rats. The incorporation in all systems proceeds linearly for at least 20 min, indicating that no activation or

TABLE 2 [^{14}C]leucine incorporation into proteins in cell-free systems consist of either total, free or bound polysomes from control and cortisone-treated rats.

Polysomes	[^{14}C]leucine incorporation (cpm/tube)	
	Control	Cortisone-treated
Total	2840	1264
Bound*	4675	2312
Free	495	528

All polysome fractions were incubated with S-165 obtained from the livers of untreated adrenalectomized rats.
*Bound polysomes were used after releasing them from membrane by deoxycholate treatment.

inactivation occurs during incubation and thus the results shown in Table 1 exactly reflect the rates of protein synthesis in vitro. The linear increase in the [^{14}C]leucine incorporation as a function of the amount of polysome in the cell-free systems shown in Fig. 2 clearly demonstrates the independence of the degree of inhibition or activation upon the amount of polysome used.

As seen in Table 2 membrane-bound polysomes are able to synthesize proteins far more actively (9-fold) than free polysomes in the untreated control animals. Cortisone treatment markedly reduces the activity of bound polysomes, whereas it does not affect that of free polysomes. These results indicate that the cortisone effect is selective and the membrane plays a decisive role in the transmission of cortisone effect to polysomes, as is the case with the cyclic AMP-mediated inhibition of cell-free protein synthesis reported by Klaipongpan et al. (4).

The mechanism by which polysomes are strikingly activated after the treatment of animals with cortisone and DBcAMP is not clear, however, the previously observed synergistic or potentiating effect of glucocorticoids on the cyclic AMP-mediated TAT induction (1,2,5) may be correlated with the cooperative effect of these drugs as shown in the present study. Since the protein syntheses by polysomes from the control rats and those treated with both drugs are equally insensitive to aurintricarboxylic acid, an inhibitor of initiation of protein synthesis (14), at low concentrations (< 100 μM) (data are not shown), it is probable that the proportion of the initiated ribosomes increases by treating animals with cortisone and DBcAMP.

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