Effect of Glucocorticoids, Insulin and a Growth Promoting Tripeptide on the Biosynthesis of Plasma Proteins in Serum-Free Hepatocyte Cultures

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Z. Naturforsch. **36 c**, 350–352 (1981);
received September 19/December 15, 1980

Rat Hepatocytes, Cultures, Plasma Proteins, Glucocorticoids, Insulin

The effect of cortisol, dexamethasone, insulin and a liver cell growth promoting tripeptide on the secretion of plasma proteins into the medium of rat hepatocytes in monolayer cultures was studied. Cortisol and dexamethasone resulted in ~ 2.5-fold increase in the fibrinogen synthesis with general supression of albumin and α-lipoprotein synthesis. On the other hand, insulin inhibited the biosynthesis of most plasma proteins except for the complement system and transferrin. Concentrations of α-lipoprotein, α-1-macroglobulin and haptoglobin were moderately elevated when the tripeptide Gly-His-Lys was applied in low concentration.

Introduction

Under pathological conditions, concentrations of a group of plasma proteins termed *acute-phase reactants* are elevated [1, 2]. Similarly, increased levels of glucocorticoids during trauma [3] were observed to affect the synthesis of hepatic RNA and proteins [4]. Numerous, but rather conflicting studies have been carried out on the effect of glucocorticoids on hepatic synthesis of proteins *in vivo* [5] as well as in suspension of hepatocytes [6] and in isolated perfused liver [7]. Therefore, it deemed essential to systematically investigate the effect of these hormones and other non-hormonal agents on the *in vitro* biosynthesis of plasma proteins in rat liver cultures.

Plasma proteins released into the culture medium of control and test hepatocyte cultures were determined by two-dimensional immunoelectrophoresis [8]. The advantage of this technique over the laborious procedures used by others is that up to 30 serum proteins can be simultaneously quantitated, 15 of which were identified in our laboratories [9].

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0341-0382/81/0300-0350 $ 01.00/0

Experimental

Animals: Specific pathogen-free male Wistar rats (150–200 g; Mus Rattus Brunnthal, Bundesrepublik Deutschland) were utilized in all experiments.

Chemicals: Insulin, cortisol and dexamethasone were obtained from Sigma GmbH (München, Bundesrepublik Deutschland). The growth promoting tripeptide Gly-His-Lys was prepared and kindly donated by Prof. Wünsch (Max-Planck-Institut für Biochemie, Martinsried, Bundesrepublik Deutschland). L-15 culture medium and polyvalent rabbit anti serum against rat serum proteins were purchased from Boehringer Mannheim (Bundesrepublik Deutschland) and Dako (Copenhagen, Denmark) respectively.

Preparation of hepatocytes and conditions of culture: Viable rat hepatocytes were prepared and maintained according to Williams [10]. Two ml of suspension containing $2-4 \times 10^5$ hepatocytes ml^{-1} in 10% fetal calf serum were incubated for four hours to allow attachment of the hepatocytes to the petri dishes. The medium was then exchanged for two ml of serum-free L-15 medium containing the desired concentration of a given agent and the antibiotic gentamin sulphate (5 µg ml^{-1}). After incubation for 18 h, the supernatant fluids of 15 petri dishes (30 ml) were collected, pooled and concentrated by pressure filtration (Amicon PM 10, Oosterhout, Holland) to a volume of 5 ml. This volume was further reduced to 0.5 ml using a Minicon B 15 concentrator.

Two-dimensional immunoelectrophoresis: Five µl of the supernatant fluid concentrate were applied to agarose gel (Behringwerke Marburg/L., Bundesrepublik Deutschland; 1% w/v in barbital buffer pH 8.6). Separation of plasma proteins was carried out according to a modified technique developed in our laboratories [9]. Polyvalent rabbit antiserum against rat serum proteins was used for the second immunoelectrophoretic run. Immunoprecipitates were characterized by differential staining methods [11]. The peak area of each precipitated protein was determined planimetrically following optical enlargement. Fibrinogen was determined according to Mancini [12] using rat anti-fibrinogen (Nordic, Holland).

Table I. Effect of added hormones to hepatocyte cultures on the secretion of plasma proteins. Percent increase/decrease in relative concentrations.

Serum Protein	[Cortisol] M			[Dexamethasone] M			[Insulin] M			Gly-His-Lys		
	5×10^{-8}	10^{-7}	5×10^{-7}	5×10^{-10}	10^{-9}	5×10^{-9}	5×10^{-10}	10^{-9}	5×10^{-9}	4 ng	20 ng	100 ng
Albumin	−30	−2.4	−8	−42	0	−20	−15	−20	−31	−9	−4	−30
α-Lipoprotein	−22	−30	−11	−33	−14	−45	−17	+10	−23	+17	0	−27
α-1-Macro-globulin	−2	−2	+15.7	−37	+20	+10	−7	−5	−23	+27	+25	−7
α-1-acid glycoprotein	†	†	†	†	†	†	†	†	†	†	†	†
α-1-Antitrypsin	−17	0	+6	−26	+11	+16	0	−10	−28	0	+2	−22
Haptoglobin	−8	+2	+8	−28	+19	+9	−5	−14	−35	+6	+18	−11
C3	−16	+14	+21	−53	−7	−37	+107	+56	0	−27	−22	+7
C3c	+20	+86	+16	−27	−11	−28	+100	+153	+98	*	*	*
Transferrin	+11	+17	+21	−35	+11	−21	+46	+10	−7	−29	−30	−25
Peak 'X'	+6	+10	+10	−13	+28	+8	+5	−8	−18	0	+4	0
Fibrinogen	+225	+249	+248	+256	+259	+241	−2	0	−1	+5	+8	0

0 No change in the relative concentration of the given plasma protein.
† Corresponding peak was depressed but was out of the immunoelectropherogram scale to allow reliable estimation.
* Peak visually not apparent.
Values given represent the mean of three experiments. The uncertainty in the percent increase/decrease synthesis, of plasma proteins of liver cell cultures prepared from different animals, ranges between 5 − 8% of the listed values.
Fibrinogen was determined according to Mancini [12].

Results and Discussion

Under our conditions of preparation of rat liver cells by collagenase perfusion, the number of hepatocytes accounted for > 95% of the total number of viable cells. Throughout this paper, the term *plasma protein synthesis* is used interchangeably with *plasma protein secretion,* as it is documented that rates of hepatic plasma protein synthesis are equal to their rates of secretion *in vitro* [13]. The percentage increase/decrease of immunoprecipitates area as a result of supplementation of the culture medium with different concentrations of a given agent are represented in Table I. In this regard, we should mention that the uncertainty in a given value in Table I did not exceed ± 1% *of the value* for different hepatocyte cultures prepared from the same rat. Notwithstanding the percentage error increased to ± 5−8% of the value due to the animal variation, values listed in Table I can be treated with confidence. Concentrations of hormones used were around the physiological values where the maximal effect of the hormone on the biosynthesis of plasma proteins may manifest itself. When the culture medium was enriched with cortisol (5×10^{-8} M), dexamethasone (5×10^{-10} M) or insulin (5×10^{-8} M; final concentrations), the synthesis of most of the plasma proteins was supressed or unaffected except for complement proteins C3 + C3c, transferrin and peak 'X' in the case of cortisol and insulin, Table I. Better attachment of hepatocytes was observed when the concentrations of the hormones were increased ten-fold, however, without a drastic change in the relative concentrations of plasma proteins secreted into the culture medium, Table I. This situation suggests that cortisol and dexamethasone have selectively affected either the synthesis or the stability of the different mRNAs in serum-free L-15 medium. It is noteworthy that albumin was the protein which was appreciably supressed at the two extreme concentrations of cortisol and dexamethasone and with all the three concentrations of insulin used, Table I. This effect may be accidental or may suggest that the hepatic synthesizing machinery used albumin translational components in building other plasma proteins, *e. g.*, transferrin, complement system C3 + C or peak 'X'. Grienger *et al.* [14] reported a similar situation in cultures of embryonic liver cells. This stimulation-inhibition effect on the *in vitro* synthesis of different plasma proteins appeared to be dependent on different hormone concentration, Table I. Both dexamethasone and cortisol stimulated liver cells were found to export fibrinogen ~ 2.5 times the rate of control cultures. The increase in fibrinogen synthesis was not affected by the increased concentration of added hormones. Also surprising is the modest *in vitro* elevation of plasma protein synthesis in the presence of 4−20 ng of Gly-His-Lys,

a concentration which, as suggested by Pickart *et al.* [15], brought about a two-fold increase in DNA and RNA biosynthesis. Higher concentration of this peptide, *i.e.*, 100 ng, led to a better attachment of hepatocytes but this was not positively relected on the protein synthesizing machinery of liver cells, Table I.

Two-dimensional immunoelectrophoresis utilized in the above manner is able to give deeper insight to possible *in vitro* mechanisms involved in hepatic protein synthesis. The hormones used in this study showed a general repressing effect with regard to plasma protein synthesis except for fibrinogen, C3c + C and peak 'X'.

Acknowledgements

We are grateful to Dr. W. Schiebel for stimulating discussion, Dr. F. J. Nordt and Ms. M. Marsh for assistance with the manuscript, and for a referee most valuable comments.

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