Effects of Hormones on the Synthesis of α_1 (Acute-Phase) Glycoprotein in Isolated Rat Hepatocytes

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(Received 18 April 1977)

Hormone effects on the synthesis of α_1 (acute-phase) glycoprotein and of albumin by isolated rat hepatocytes in suspension were examined. Insulin, glucagon, cortisol, somatotropin (bovine growth hormone) and tri-iodothyronine were added to achieve physiological concentrations in the medium [Jeejeebhoy, Ho, Greenberg, Phillips, Bruce-Robertson & Sodtke (1975) *Biochem. J.* 146, 141–155]. After periodic additions, there were increases (compared with values for non-hormone-treated suspensions) in the concurrent absolute syntheses of α_1 (acute-phase) glycoprotein and of albumin. Trends were detectable after 24 h, and significant increases were demonstrated after 48 h of incubation (219 and 119% respectively of control values). Manipulation of hormones, by omission from the mixture or by addition of only one or two hormones in various combinations, indicated that for α_1 (acute-phase) glycoprotein (which may be representative of some other acute-phase proteins), cortisol was one of the most important hormones involved in the stimulation of synthesis, with glucagon enhancing the effect of cortisol but not being stimulatory by itself. Addition of actinomycin D inhibited this stimulation, suggesting that cortisol might have acted through promotion of RNA synthesis. For albumin, cortisol alone did not stimulate synthesis, but its absence from a hormone mixture significantly decreased synthesis compared with that observed with the complete hormone mixture. Our findings support the possibility that following tissue injury, synthesis of α_1 (acute-phase) glycoprotein may be stimulated by the hormonal response to this injury (which response includes elevated blood concentrations of cortisol and glucagon).

Injury, infection, tissue necrosis and tumour growth are all associated with an increased concentration of a group of plasma proteins referred to as acute-phase proteins (Gordon, 1970, 1976), a representative example being α_1 (acute-phase) glycoprotein (Darcy, 1964, 1966). By contrast, the concentration of albumin is often decreased under the same circumstances (Gordon, 1973). The increase or decrease in the blood concentration of plasma protein may be the result of several factors, including changes in synthesis and/or catabolism. However, it is known that the increased blood concentration of acute-phase proteins during the above conditions is indeed associated with increased synthesis, and that correspondingly synthesis of albumin may be decreased under the same conditions (Williams, 1970). Since one set of factors well known to be altered during injury is the concentration of various hormones (Batstone *et al.*, 1976), the object of the present studies was to attempt to clarify the manner in which certain hormonal changes may influence the synthesis of these two proteins *in vitro*. In the burned patients studied by Batstone *et al.* (1976), cortisol concentration in plasma was dramatically increased

on the first day of the acute phase that followed injury, and that of glucagon rose about 2 days later. Hence it seemed of interest to examine the effect of these hormones (known to be increased in one form of injury, namely burns) on plasma protein synthesis. We have found that cortisol enhances the synthesis of albumin (and of fibrinogen) *in vivo* (Jeejeebhoy *et al.*, 1973). Interpretation of manipulation *in vivo*, however, is subject to the problems induced by a hormone's influence on several concurrent processes, including amino acid mobilization (Munro, 1964), thus making it difficult to define its specific effect on the liver. By contrast with this enhanced synthesis with cortisol *in vivo*, there is a decreased rate of synthesis of albumin (and of transferrin) with glucagon *in vitro*, in the perfused liver (Tavill *et al.*, 1973). These perfused-liver studies, however, were carried out for only 4–6 h, not long enough to be certain that the effects demonstrated were really representative of those of glucagon on the hepatocyte *in vivo*, for John & Miller (1969) have shown that it takes a prolonged period after administration of a hormone (more than 8 h) before its effect is demonstrated *in vitro*. Similar findings to those by John & Miller (1969) were obtained by

Crane & Miller (1974) and ourselves (Jeejeebhoy *et al.*, 1975). Our present study was designed therefore to determine the effect, on the concurrent synthesis of α_1 (acute-phase) glycoprotein (Darcy, 1964, 1966) and albumin, of insulin and cortisol, alone and in combination with tri-iodothyronine *O*-(4-hydroxy-3-iodophenyl)-3,5-di-iodo-L-tyrosine, somatotropin (bovine growth hormone) and glucagon, administered in quantities that produce approximately physiological concentrations. Also the effects of hormonal changes in the presence of actinomycin D, an inhibitor of RNA synthesis, were studied to elucidate the mechanisms involved.

Materials and Methods

Hepatocyte suspension system

The preparation and incubation of hepatocytes for 48 h was carried out as described previously (Jeejeebhoy *et al.*, 1975), except that cells, in the 2.0 ml samples taken at intervals, were disrupted by sonication instead of freezing and thawing (Sonifier Cell Disruptor, Heat Systems-Ultrasonics Inc., Plainview, Long Island, NY, U.S.A.; 20 kHz, 30 W for 1 min).

Syntheses of α_1 (acute-phase) glycoprotein and albumin

The amount of each protein was determined by the solid-phase radioimmunoassay of Askenase & Leonard (1970) as described previously (Jeejeebhoy *et al.*, 1975). As before, net increases (compared with values observed at the start of culture) in the amounts of α_1 (acute-phase) glycoprotein and albumin in timed, homogeneous and sonicated samples, were taken as synthesis. These values reflect synthesis rather than release, because they represent an increase in the total mass of specific intra- and extra-cellular protein. The increases in the given plasma protein were related to the average wet weight of cells over each successive time period. Furthermore, in separate experiments (results not included) incorporation of [3 H]valine into protein was observed after the period of incubation, and this incorporation could be suppressed with cycloheximide. Unless otherwise stated, values for synthesis are those observed at 48 h and are presented as mg/g wet wt. of hepatocytes.

Proteins and antibodies used

Albumin was prepared as described previously (Ho *et al.*, 1974). The α_1 (acute-phase) glycoprotein (Darcy, 1964, 1965) was prepared by the method of Gordon & Dykes (1972), except for omission of the third stage of isoelectric focusing, and was kindly given to us by Dr. A. Hugh Gordon of the National Institute for Medical Research, London, U.K. He also generously gave a specific antiserum (his no.

V/4/AS1) that he raised in a rabbit by the following method: 1% α_1 -globulin in complete Freund's adjuvant was injected subcutaneously at many (20) sites. This injection was repeated after 3 and 7 weeks. Ten days after the last injection, the rabbit was bled and whole serum separated. Antisera for rat albumin had been prepared previously by ourselves (Jeejeebhoy *et al.*, 1975) using a similar method. The antibodies consisted of partially purified γ -globulin fractions of these sera. The precipitates resulting from 30% saturation of the sera with $(\text{NH}_4)_2\text{SO}_4$ were dialysed and eluted from the anion-exchange resin DEAE-Sephadex A-50 (Pharmacia, Montreal, Que., Canada) with 0.03 M-sodium phosphate buffer, pH 7.00, and the eluate was concentrated with a Diaflo UM-10 membrane. The specificity of these antibodies was demonstrated by showing that in the radioimmunoassay system there was no cross-reaction with the horse serum in the medium or with a variety of other rat plasma proteins separated by the methods of Ho *et al.* (1974).

Experimental procedure

In all studies, unless otherwise stated, cells from the same batch of hepatocytes were divided into three equal portions and incubated in paired control and test spinner flasks, thus allowing for the variability between batches of cells. [This approach has proved (Jeejeebhoy *et al.*, 1975) to be a sensitive means of demonstrating the effect of manipulation in the test incubation.] Hormones dissolved in medium were added as a group or singly in amounts calculated to give the suspension concentrations noted below at 6, 12 and 18 h of incubation. At 24 h the last addition of hormones was made at concentrations of four times the concentrations in 6 h additions, the maximum volume so added being 1.2 ml, or approx. 1% of the total volume of suspension remaining at that time. The hormones, and their final concentrations (per ml of the medium), were: insulin, 0.8 ng; glucagon, 0.75 ng; cortisol, 0.25 μg ; somatotropin, 0.5 μg ; and tri-iodothyronine, 0.09 μg .

In the first set of experiments (I), control cells were compared with cells receiving the group of hormones. Concurrently a third batch of cells was incubated with the same hormone mixture from which cortisol had been omitted. In the second set of experiments (II), one incubation consisted of control cells and was compared with cells treated with insulin alone and with cells treated with cortisol alone. A third set (III) consisted of one group treated with cortisol plus somatotropin, a second with cortisol plus glucagon, and a third with cortisol plus tri-iodothyronine. A fourth set (IV) compared a group of control cells with those treated with glucagon alone. A final set of experiments (V) was carried out to compare control cells with those treated by the addition of cortisol

with or without glucagon and those similarly treated, but with actinomycin D added (to a concentration of 0.5 µg/ml of medium after 24 h of incubation).

Statistics

The results of a given experimental procedure were analysed by both the paired and unpaired Student's *t* test (Hewlett-Packard 9810A Calculator, Model 10 Statistics Programs 5-5 and 5-6). Results from different experiments were compared by using the unpaired Student's *t* test. The absolute synthesis of a protein (over the time period shown) is given as the mean number of mg of the protein produced per g of hepatocytes $\pm$ S.E.M. (*n*).

Results

Experiment I

The observations for α_1 (acute-phase) glycoprotein are shown in Table 1. The addition of the hormone mixture (insulin, glucagon, cortisol, triiodothyronine and somatotropin) more than doubled the synthesis of this protein ($P < 0.02$) over a 48 h period. Synthesis at this time (Table 1) was 0.85 ± 0.20 (8) and 1.85 ± 0.41 (8) mg/g of hepatocytes in control

and hormone-treated cells respectively. The difference is striking when synthesis from 0 to 24 h is compared with that from 24 to 48 h of incubation. During the second 24 h period the synthesis of α_1 (acute-phase) glycoprotein in the control is less, whereas that for the hormone-treated cells is more, than for the respective preceding 24 h period. Albumin synthesis in contrast increased above the control value by only 19% ($P < 0.01$); after 48 h of incubation it was 5.59 ± 0.68 (7) in controls and increased to 6.68 ± 0.54 (7) with hormone treatment.

The effects of omitting cortisol from the hormone mixture are shown in Table 1. The omission of cortisol was associated with the following alterations in synthesis (mg/g of hepatocytes) at 48 h. The values of 0.64 ± 0.14 (4) and 5.39 ± 0.68 (4) for α_1 (acute-phase) glycoprotein and albumin respectively are decreased ($P < 0.05$) from those for cells treated with the complete hormone mixture, but are indistinguishable from those for controls.

Experiment II

The results are shown in Table 2. When single hormones were added to the incubation mixture, cortisol alone increased the mean 48 h synthesis of α_1 (acute-phase) glycoprotein by about 81% over that

Table 1. Effect of complete hormone mixture* and of hormone mixture without cortisol* on the synthesis (mg/g of hepatocytes) of α_1 (acute-phase) glycoprotein and of albumin

Time of incubation (h) ...	α_1 (Acute-phase) glycoprotein synthesis				Albumin synthesis 48
	6	12	24	48	
Control [mean $\pm$ S.E.M. (<i>n</i>)]	0.16 ± 0.036 (8)	0.37 ± 0.13 (8)	0.63 ± 0.18 (8)	0.85 ± 0.20 (8)	5.59 ± 0.68 (7)
Hormone mixture [mean $\pm$ S.E.M. (<i>n</i>)]	0.22 ± 0.069 (8)	0.29 ± 0.073 (8)	0.70 ± 0.16 (8)	1.85 ± 0.41 (8)	6.68 ± 0.54 (7)
Hormone mixture minus cortisol [mean $\pm$ S.E.M. (<i>n</i>)]	0.12 ± 0.031 (3)	0.25 ± 0.059 (4)	0.48 ± 0.12 (4)	0.64 ± 0.14 (4)	5.39 ± 0.68 (4)

* For details of hormone mixture with and without cortisol see the text.

Table 2. Effect of insulin* and of cortisol* on the synthesis (mg/g of hepatocytes) of α_1 (acute-phase) glycoprotein and of albumin

Time of incubation (h) ...	α_1 (Acute-phase) glycoprotein synthesis				Albumin synthesis 48
	6	12	24	48	
Insulin alone [mean $\pm$ S.E.M. (<i>n</i>)]	0.13 ± 0.05 (5)	0.22 ± 0.08 (4)	0.38 ± 0.09 (5)	0.66 ± 0.13 (5)	5.14 ± 0.68 (5)
Cortisol alone [mean $\pm$ S.E.M. (<i>n</i>)]	0.13 ± 0.05 (6)	0.20 ± 0.07 (5)	0.43 ± 0.09 (6)	1.12 ± 0.18 (6)	6.33 ± 0.81 (6)
Control (no hormone) [mean $\pm$ S.E.M. (<i>n</i>)]	0.14 ± 0.04 (6)	0.21 ± 0.06 (5)	0.40 ± 0.11 (6)	0.62 ± 0.15 (6)	5.98 ± 0.61 (6)

* The amount of single hormone added and times of addition are similar to those given for the hormone mixture; see the text.

of the control (no hormone), the respective values being 1.12 ± 0.18 (6) and 0.62 ± 0.15 (6) ($P < 0.05$). In contrast, for albumin synthesis, cortisol alone made no significant difference, the values with and without it being 6.33 ± 0.81 (6) and 5.98 ± 0.61 (6).

Experiment III

The results are set out in Table 3. When combinations of hormones were used it became clear that for the glycoprotein, the combined addition of glucagon and cortisol resulted in a 22% higher synthesis [i.e. 2.42 ± 0.29 (6)], significantly greater than that observed for the combined addition of cortisol with either somatotropin [1.93 ± 0.34 (6) ($P < 0.05$)] or tri-iodothyronine [1.98 ± 0.21 (6) ($P < 0.01$)]. However, the highest synthesis for albumin was seen with tri-iodothyronine in combination with cortisol, when the result [9.53 ± 0.45 (6)] was greater than that for the combined addition of cortisol with either somatotropin [7.61 ± 0.16 ($P < 0.01$)] or glucagon [8.11 ± 0.071 (6) ($P < 0.05$)].

Experiment IV

Glucagon alone had no effect on α_1 (acute-phase) glycoprotein synthesis (results not shown).

Experiment V

In the test flasks into which actinomycin D was injected after 24 h of incubation of the cells with cortisol (or cortisol plus glucagon), the expected elevation of α_1 (acute-phase) glycoprotein synthesis did not occur, although this was observed, as described above, in the control (hormone-treated) flasks between 24 and 48 h of incubation. The respective values were 0.39 ± 0.04 (3) (actinomycin D added to hormone) and 0.62 ± 0.06 (3) (hormone only throughout) ($P < 0.05$). The albumin synthesis after 48 h incubation of these cells after treatment with cortisol (or cortisol plus glucagon) was indistinguishable from that in controls (no hormone), and the further addition of actinomycin D to the hormone had no detectable effect on albumin synthesis (results not shown).

Table 3. Effect of hormone combinations* on the synthesis (mg/g of hepatocytes) of α_1 (acute-phase) glycoprotein and of albumin

Time of incubation (h) ...	α_1 (Acute-phase) glycoprotein synthesis				Albumin synthesis 48
	6	12	24	48	
Cortisol plus somatotropin [mean $\pm$ S.E.M. (n)]	0.080 ± 0.028 (6)	0.18 ± 0.021 (6)	0.59 ± 0.074 (6)	1.93 ± 0.34 (6)	7.61 ± 0.16 (6)
Cortisol plus glucagon [mean $\pm$ S.E.M. (n)]	0.057 ± 0.0088 (6)	0.20 ± 0.031 (6)	0.65 ± 0.065 (6)	2.42 ± 0.29 (6)	8.11 ± 0.071 (6)
Cortisol plus tri-iodothyronine [mean $\pm$ S.E.M. (n)]	0.058 ± 0.0060 (6)	0.23 ± 0.020 (6)	0.55 ± 0.052 (6)	1.98 ± 0.21 (6)	9.53 ± 0.45 (6)

* Amounts of each single hormone added, in the combinations used, and the times of addition are the same as those given under 'Experimental procedure' for the hormone mixture.

Table 4. Summary of effects of hormones* on the 48 h synthesis (mg/g of hepatocytes) of α_1 (acute-phase) glycoprotein and of albumin

	α_1 Glycoprotein synthesis	Albumin synthesis	α_1 Glycoprotein synthesis (% of albumin)
Control	0.85	5.59	15.2
Control	0.62	5.98	10.4
All five hormones*	1.85	6.68	27.7
Cortisol plus glucagon	2.42	8.11	29.8
Cortisol plus tri-iodothyronine	1.98	9.53	20.8
Cortisol plus somatotropin	1.93	7.61	25.4
Cortisol only	1.12	6.33	17.7
Insulin only	0.66	5.14	12.8
Glucagon plus tri-iodothyronine plus somatotropin plus insulin	0.64	5.39	11.9

* Details of dosage and times of addition are given under 'Experimental procedure'.

Comparison of results between experimental groups

Results (Table 4) were compared to determine whether the addition of somatotropin, glucagon or tri-iodothyronine, each in combination with cortisol, increased protein synthesis above that observed with cortisol alone. For α_1 (acute-phase) glycoprotein synthesis, the results show that the addition of glucagon to cortisol resulted in a greater (and more significant) increase ($P < 0.01$) in the 48 h synthesis than for the addition of tri-iodothyronine ($P < 0.05$), compared with cortisol alone. For albumin, the mean synthesis after 48 h of incubation in cells treated with cortisol alone was 6.33 (indistinguishable from control values). However, this was significantly increased ($P < 0.05$) by the combination of glucagon with cortisol, and even more increased ($P < 0.01$) by the addition of tri-iodothyronine with cortisol, the respective values being 8.11 and 9.53. However, the addition of somatotropin to the cortisol did not change synthesis significantly from that observed with cortisol alone. From Table 4 it is clear that the complete hormone mixture and the combination of cortisol with either tri-iodothyronine, glucagon or somatotropin were especially effective in enhancing the synthesis of α_1 (acute-phase) glycoprotein above that for albumin, compared with the control.

Discussion

The effect of hormones on hepatocytes has received attention with the observation that insulin and glucagon may influence hepatic regeneration and be required for optimum survival of these cells (Starzl *et al.*, 1973). An important differentiated hepatocyte function, the maintenance of plasma protein synthesis, requires cortisol in the isolated perfused liver (John & Miller, 1969). Supporting this finding are studies *in vivo* (Jeejeebhoy *et al.*, 1973) showing that cortisol directly enhances synthesis of albumin, fibrinogen and transferrin when observations are made 24 h after administering the hormone. Furthermore these studies showed that the short-term (3 h) effect of cortisol differed from the later (24 h) effect, and that amino acid supplementation influenced the result. However, both the perfused-liver study (John & Miller, 1969) and the study *in vivo* (Jeejeebhoy *et al.*, 1973) used pharmacological doses of cortisol. In isolated hepatocytes, Crane & Miller (1974) showed that cortisol and insulin are required for maximum fibrinogen synthesis. However, albumin synthesis in their studies stopped after 17 h of incubation. Jeejeebhoy *et al.* (1975) showed that cortisol in a hormone mixture [somatotropin (bovine), tri-iodothyronine and glucagon] minus insulin enhanced fibrinogen synthesis, and that cortisol, with insulin in the mixture, enhanced albumin synthesis. However, evaluation of the effects of single hormones and

detailed studies with various combinations of hormones was not carried out. There is even less information (Darcy, 1965) on the effect of hormones on the synthesis of α_1 (acute-phase) glycoprotein.

The results in the present paper show that a hormone mixture significantly enhanced synthesis of α_1 (acute-phase) glycoprotein and albumin after 48 h, the former effect being more pronounced. By omitting single hormones from the mixture it was shown that cortisol was an important hormone for stimulating synthesis of α_1 (acute-phase) glycoprotein, with glucagon enhancing the effect of cortisol. Since blood concentrations of both these hormones definitely increase after one type of injury (burns), it is possible that the post-injury increase in plasma concentration of α_1 (acute-phase) glycoprotein may well be the result of stimulation of the latter's synthesis by this hormonal response to injury, among other factors. Because the addition of actinomycin D inhibited the considerable enhancement of synthesis of α_1 (acute-phase) glycoprotein by cortisol, we suggest that increased RNA synthesis is a prerequisite for the increased synthesis of this protein. Since actinomycin D had no effect on albumin synthesis, it may be that, for this protein, cortisol operates through a different mechanism in stimulating synthesis. In this connexion it may be pertinent that, unlike the synthesis of α_1 (acute-phase) glycoprotein, that of albumin was not stimulated by cortisol alone, although cortisol was required (in conjunction with other hormones) for such stimulation to occur.

We thank the Medical Research Council of Canada for continuing financial support for this work (grant no. MT. 3204) and the Toronto General Hospital Foundation for the interim main salary support of R. M. We most especially thank Dr. A. Hugh Gordon, of the National Institute for Medical Research, Mill Hill, London, U.K., for his generosity and kindness in providing α_1 (acute-phase) glycoprotein and rabbit antiserum to it, and for his various suggestions. Again we thank Janet Chrupala for her expert typing.

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