The effects of glucocorticoids on insulin-stimulated lipogenesis in primary cultures of rat hepatocytes

John M. AMATRUDA,* Susan A. DANAHY and Cecilia L. CHANG
Department of Medicine, Division of Endocrinology and Metabolism, University of Rochester School of Medicine, 601 Elmwood Avenue, Rochester, NY 14642, U.S.A.

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We used primary cultures of rat hepatocytes to evaluate the effects of glucocorticoids on insulin-responsive hepatic lipogenesis. The data indicate that hepatocytes incubated for 20h with dexamethasone (0.1µM) alone are profoundly resistant to the ability of insulin to stimulate lipogenesis acutely. In contrast, primary cultures of hepatocytes incubated with dexamethasone plus insulin are hyper-responsive to the ability of insulin to stimulate lipogenesis chronically. This potentiation of insulin action by a glucocorticoid occurs at physiological concentrations of the two hormones. Exposure to dexamethasone plus insulin for more than 4h is required for the two hormones to enhance insulin action either by overcoming the insulin resistance induced by dexamethasone alone or by stimulating insulin action induced by insulin alone. Despite the marked potentiation of insulin action, hepatocytes exposed to dexamethasone plus insulin are less sensitive to insulin, as demonstrated by a shift to the right in the dose–response curve for insulin-stimulated lipogenesis. The resistance of hepatocytes to the acute effects of insulin after exposure to dexamethasone alone and the potentiation of insulin action and decreased sensitivity to insulin after exposure to insulin plus dexamethasone are all mediated by post-insulin-binding events. These studies demonstrate potentiation of insulin action in the liver by physiological concentrations of glucocorticoids and may have physiological significance for the regulation of normal hepatic lipogenesis, for the hyperlipidaemia observed with the pharmacological use of glucocorticoids, and for disease states in man associated with hyperinsulinaemia and hypercortisolism.

Increased concentrations of glucocorticoids in humans and animals are associated with enhanced hepatic rates of lipogenesis and increased hepatic and plasma lipids (Baker et al., 1948; Steinberg et al., 1952; Soffer et al., 1961; Stern et al., 1973; Casaretto et al., 1974; Reaven et al., 1974; Diamant & Shafrir, 1975; Bagdade et al., 1976a,b; Kirk et al., 1976). In addition, physiological concentrations of glucocorticoids in conjunction with insulin are required for the enhanced lipogenesis associated with refeeding after starvation (Berdanier & Shubeck, 1979; Bouillon & Berdanier, 1980). The relative roles of these two hormones in this hyperlipogenic response are unknown. It has been suggested that the increased insulin concentrations associated with glucocorticoid excess, or a synergism between glucocorticoids and insulin, as in refeeding, may mediate the enhanced hepatic lipogenesis (Kirk et al., 1976; Berdanier & Shubeck, 1979). If so, this would represent a situation of differential responsiveness to glucocorticoids, since glucocorticoids induce insulin resistance for some biological responses in the liver when administered in vivo or in vitro (Caro & Amatruda, 1982). Although the exact mechanism of this insulin resistance is also unknown, studies in our laboratory with freshly isolated hepatocytes from glucocorticoid-treated animals and primary cultures of hepatocytes exposed to dexamethasone (0.1µM) indicate that the insulin resistance in the liver with regard to amino-isobutyrate uptake is due to post-binding mechanisms (Caro & Amatruda, 1982). The direct effects of glucocorticoids on the liver are to increase insulin binding and to induce resistance to the ability of

* To whom reprint requests should be addressed.
insulin both to stimulate amino acid uptake and to
down-regulate its receptors (Caro & Amatruda,
1982).

To define further the effects of glucocorticoids on
another metabolic process in the liver and to
establish the nature of the interrelationships between
insulin and glucocorticoids on this process, we have
evaluated lipogenesis in primary cultures of hepatocytes
exposed to dexamethasone and insulin, separ-
ately or together. Primary cultures of hepatocytes
were used because they allow us to evaluate the
effects of these hormones singly and combined
without the complicating changes in substrate and
hormonal milieu that occur in vivo.

The data indicate that dexamethasone alone leads
to an insulin-resistant state with regard to lipo-
genesis much the same as we have previously
demonstrated for amino acid uptake (Caro &
Amatruda, 1982). The combination of dexametha-
sone plus insulin, however, leads to a marked
enhancement of insulin-stimulated lipogenesis. Thus,
under conditions where insulin is present, gluco-
corticoids are permissive for stimulation of lipo-
genesis by insulin, whereas under conditions
insulin lack glucocorticoids induce or aggravate
insulin resistance.

Experimental

Male Sprague–Dawley rats (200–300g) fed ad
libitum were used for all experiments. The isolation
of liver cells and preparation of primary cultures of
rat hepatocytes on collagen-coated plates in serum-
free medium was as previously described (Caro &
Amatruda, 1980a,b; Cech et al., 1980; Amatruda
et al., 1982). Any additions to the culture medium
were added at the time of the first change of medium,
3–4h after plating. Insulin was added again 16h later
and 4h after this the cells were washed at 37°C with
Hanks–Hepes [4-(2-hydroxymethyl)-1-piperazine-
ethanesulphonic acid] buffer, pH7.4, containing
8mm-glucose and albumin (10mg/ml).

To measure lipid synthesis, [14C]acetate (5mm;
2.2μCi/ml) was added to the culture plates con-
taining the above Hanks–Hepes buffer and the
incubation continued at 37°C for 2h. The plates
were then washed in cold phosphate-buffered saline,
PH7.4, and the cellular lipids were extracted by the
method of Bligh & Dyer (1959) as previously
reported (Amatruda et al., 1982). In some experi-
ments lipid synthesis was measured by the incor-
poration of 3H2O (0.6mCi/ml) plus 5mm unlabelled
acetate (Amatruda et al., 1982). Binding of 125I-
labelled insulin was measured as previously reported
(Caro & Amatruda, 1980a,b; Cech et al., 1980;
Amatruda et al., 1982) and expressed as specific
binding. Dexamethasone was dissolved in water,
filtered on Whatman no. 1 filter paper, and the
concentration was measured spectrophotometrically
at 250nm. A stock solution of 10μM was prepared
and diluted in culture medium.

3H2O (1Ci/g) and [1-14C]acetate, sodium
salt (1.9Ci/mol), were purchased from New England
Nuclear, Boston, MA, U.S.A.; crude collagenase
(CLIS II; batch no. 40-162P; 138units/mg) was
from Worthington Biochemical Corp., Freehold, NJ,
U.S.A.; dexamethasone and all amino acids were
from Sigma Chemical Co., St. Louis, MO, U.S.A.; and
serum-free medium (Waymouth's MB 752/1) and
bovine albumin (fraction V) were from Grand
Island Biological Co., Grand Island, NY, U.S.A.
Crystalline pig insulin was kindly provided by Dr.
Ronald Change of Eli Lilly and Co., Indianapolis,
IN, U.S.A.

Results

Previous studies in our laboratory demonstrated
that the exposure of primary cultures of hepatocytes
to glucocorticoids leads to insulin resistance with
regard to amino acid uptake (Caro & Amatruda,
1982). The present studies evaluating lipogenesis
resulted in similar findings. The acute (2h) lipogenic
response to insulin of primary cultures of hepatocytes
from normal rats was evaluated after a 16h exposure
to dexamethasone (0.1μM). These data are
illustrated in Fig. 1 and demonstrate a profound

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**Fig. 1.** *Acute lipogenic response to insulin of primary cultures of rat hepatocytes incubated for 20h with no additions (O) or in the presence of dexamethasone (●)*

For full details see the Experimental section. The
data represent means ± S.E.M. for four separate
experiments. Basal lipogenesis measured in the
absence of insulin for 2h is 12.3 ± 1.9 and
13.7 ± 3.5nmol of [1-14C]acetate incorporated into
lipids/mg of cellular protein in control and dexa-
methasone-treated cultures respectively.
state of insulin resistance after the exposure of hepatocytes to dexamethasone as compared with control cultures. This decrease in responsiveness is significant at all insulin concentrations \((P<0.05 \text{ to } P<0.025)\). Hepatocytes exposed to dexamethasone have no detectable lipogenic response to insulin at hormone concentrations of 0.1 and 1 nm. At higher concentrations (10 and 100 nm) the insulin response of cultures treated with dexamethasone is only 8% and 4% that of control cultures respectively (Fig. 1).

To validate these findings and to determine if alterations in substrate pool sizes could be affecting our interpretation of the insulin responsiveness of dexamethasone-treated cells, we evaluated the acute lipogenic response to insulin, using \(^3\)H\(_2\)O and unlabelled sodium acetate (5 mM). In two experiments the acute lipogenic response to insulin (10 nM) in cultures exposed for 20 h to dexamethasone (0.1 \(\mu\)M) was only 35% of that of control cultures. These data indicate that resistance to the acute effects of insulin are present when utilizing both \(^3\)H\(_2\)O and [1-\(^14\)C]acetate as substrates, but that under these conditions dexamethasone may also be having an effect on the pool size of lipogenic precursors.

Since the lipogenic response to insulin is so low in dexamethasone-treated cells (Fig. 1), a concentration of insulin eliciting a half-maximal response (ED\(_{50}\)) cannot be accurately determined. However, in the control cultures the ED\(_{50}\) for insulin is 0.4 nm, which is similar to that previously reported from our laboratory (Caro & Amatruda, 1980a,b; Cech et al., 1980; Amatruda et al., 1982). Under the conditions of the present studies, insulin binding is increased, as we have previously reported (results not shown). (Caro & Amatruda, 1982), indicating that the dexamethasone-induced insulin resistance is due to post-binding mechanisms.

In contrast with the acute response to insulin, when primary cultures of hepatocytes are incubated in the presence of dexamethasone (0.1 \(\mu\)M) plus various concentrations of insulin for 20 h lipogenesis is markedly enhanced (Fig. 2). At 10 nm-insulin the enhancement of lipogenesis by dexamethasone plus insulin is 3.6-fold (4.9-fold at 100 nm-insulin) greater than that observed with insulin alone (Fig. 2). This enhancement of lipogenesis is significant at all insulin concentrations \((P<0.05 \text{ to } P<0.0125 \text{ by paired } t \text{ test})\). These data were also confirmed with \(^3\)H\(_2\)O. At 10 nm-insulin plus 0.1 \(\mu\)M-dexamethasone, insulin-stimulated lipogenesis was enhanced 4.6-fold over that observed with insulin alone \((P<0.005)\). Although this enhancement of lipogenesis occurs at physiological concentrations of insulin, dexamethasone also has a significant effect on insulin sensitivity. The ED\(_{50}\) concentrations of insulin are 0.66 and 2.1 nm in the control and dexamethasone-treated cells respectively \((P<0.025 \text{ by paired } t \text{ test})\). This significant decrease in insulin sensitivity (Fig. 2) in the presence of an increase in insulin binding in previously published studies (Caro & Amatruda, 1982) again indicates that post-binding mechanisms can regulate both the sensitivity and responsiveness to insulin (Amatruda et al., 1982). In addition, the present data indicate that these two parameters can be regulated in opposite directions, i.e. cells treated with dexamethasone plus insulin have a decrease in sensitivity and an increase in responsiveness to insulin.

To determine the dose–response relationship for the enhancement of lipogenesis by dexamethasone, we incubated primary cultures of hepatocytes for 20 h in the presence of various concentrations of dexamethasone (1 nm–0.1 \(\mu\)M) and a fixed concen-
tration of insulin (10 nm). Lipogenesis was then evaluated. A significant glucocorticoid effect was observed at 1 nm-dexamethasone (Fig. 3). At this concentration, dexamethasone in the presence of insulin led to a 25% enhancement of lipogenesis compared with insulin alone (P < 0.05 by paired t test). The enhancement by dexamethasone of insulin-stimulated lipogenesis was 109% (P < 0.005) and 143% (P < 0.001) at 10 nm- and 0.1 µM-dexamethasone respectively, with an EBD of 5.5 nm-dexamethasone. In these experiments, dexamethasone had no effect on basal lipogenesis, i.e. in the absence of insulin. Assuming a glucocorticoid potency 37.5 times that of cortisol, a significant glucocorticoid effect is observed at a dexamethasone concentration equivalent to 1.5 µg of cortisol/100 ml (1 nm-dexamethasone), a half-maximal effect at a dexamethasone concentration equivalent to 8.25 µg of cortisol/100 ml and a maximal increase in effect at a dexamethasone concentration equivalent to 15 µg of cortisol/100 ml, well within the physiological range for basal plasma cortisol concentrations in non-stressed man.

We next evaluated the time course of the dexamethasone effect on lipogenesis. Primary cultures of hepatocytes were incubated with insulin alone for 0, 2, 4, 12 and 20 h and lipogenesis from [1-14C]acetate was evaluated over a 2 h period. As illustrated in Fig. 4, insulin action is maximal by 2 h of preincubation (P < 0.1 for 2 versus 4 h). With more prolonged preincubation with insulin, no further increase in insulin’s effect was observed. In additional experiments, primary cultures of hepatocytes were incubated with dexamethasone for 20 h and insulin was added for the full 20 h incubation or for the final 12, 4, 2 or 0 h. Lipogenesis was then evaluated from [1-14C]acetate. As illustrated in Fig. 4, as compared with insulin alone, cultures incubated with dexamethasone for 20 h were resistant to insulin’s effect on lipogenesis for 2 and 4 h (P < 0.01). By 12 h insulin is able to overcome this resistance, and insulin-stimulated lipogenesis is enhanced 2.2-fold in the presence of dexamethasone for 20 h and insulin for 12 h. When both insulin and dexamethasone are included for 20 h there is a 3.4-fold enhancement of insulin action. Thus dexamethasone-induced insulin resistance is present only within 4 h of insulin exposure. After this time, the two hormones act together to enhance insulin responsiveness. In primary cultures of hepatocytes incubated with insulin for 20 h, dexamethasone has no effect on insulin-stimulated lipogenesis for the first 4 h of exposure to dexamethasone, i.e. cultures are incubated with 10 nm-insulin for 20 h and 0.1 µM-dexamethasone is added at 16 h and 18 h of incubation or at 4 and 2 h before measurement of [14C]acetate incorporation into lipids (Fig. 4). When dexamethasone is added for the final 12 h and the entire 20 h of culture, the data are identical with those for the cultures in which insulin is added after dexamethasone. Thus exposure to insulin plus dexamethasone must be for greater than 4 h for the two hormones to enhance insulin action either by overcoming the insulin resistance induced by dexamethasone.

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Fig. 3. Lipogenesis in primary cultures of hepatocytes after a 20 h exposure to various concentrations of dexamethasone alone (●) or plus 10 nm-insulin (○). Lipogenesis is measured from [1-14C]acetate as described in the Experimental section. The data represent means ± S.E.M. for three separate experiments.
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Fig. 4. Time course of the effect of insulin and dexamethasone plus insulin on lipogenesis

Primary cultures of hepatocytes were incubated with insulin (10 nM) alone for 0, 2, 4, 12 or 20 h and lipogenesis was measured over 2 h (●), or with dexamethasone (0.1 µM) for 20 h and insulin (10 nM) for the last 0, 2, 4, 12 or 20 h of the dexamethasone incubation (▲), or with insulin (10 nM) for 20 h and dexamethasone (0.1 µM) for the last 0, 2, 4, 12 or 20 h of the insulin incubation (□). The data represent means of three experiments. Basal lipogenesis in the absence of any additions is 15.7 ± 2.3, 16.4 ± 2.8 and 16.0 ± 1.1 nmol/2 h per mg of protein in the three groups of experiments respectively. Panel (a) represents the increase in lipid synthesis above basal owing to insulin and dexamethasone plus insulin. Panel (b) represents the same data expressed as a percentage of the maximal effect observed in panel (a).

Discussion

The role of glucocorticoids in the regulation of hepatic lipogenic enzymes and lipogenesis has been studied in intact animals and in the perfused rat liver (Altman et al., 1951; Diamant & Shafrir, 1975; Kirk et al., 1976). This effect of glucocorticoids was first demonstrated by Altman et al. (1951), who showed that pharmacological amounts of insulin and glucocorticoids act synergistically to enhance lipogenesis in the perfused liver. Subsequent studies indicated that the administration of glucocorticoids to rats increases the activity of hepatic acetyl-CoA carboxylase and fatty acid synthetase as well as lipogenesis from [1-14C]acetyl-CoA (Diamant & Shafrir, 1975). These effects were not present in alloxan-diabetic rats, suggesting that insulin is a necessary mediator of the glucocorticoid effect. Additional studies in starved-refed rats indicate that the increase in glucose 6-phosphate dehydrogenase and malic enzyme activities which is present in normal rats is not demonstrable in adrenalectomized, streptozotocin-diabetic and adrenalectomized–diabetic rats (Berdanier & Shubeck, 1979). Treatment with glucocorticoids, insulin, and both hormones in the respective groups restores the hepatic enzyme overshoot associated with refeeding (Berdanier & Shubeck, 1979). From those studies it has been suggested that insulin may be responsible for the regulation of substrate flux into lipogenic pathways, and glucocorticoids and insulin may be responsible for enzyme protein synthesis de novo (Berdanier & Shubeck, 1979). In addition, in perfused livers from adrenalectomized rats, lipogenesis from 3H2O and 14C-labelled precursors is decreased (Kirk et al., 1976). Cortisol addition to the perfusion has no stimulatory effect on lipogenesis, whereas fatty acid synthesis is restored to normal in perfused livers from adrenalectomized rats within 5 h after treatment of the animal with cortisol in vivo. These studies suggested that both insulin and glucocorticoids are necessary for normal hepatic lipogenesis. Glucocorticoid excess in both animals and man leads to hepatic lipid accumulation (Baker et al., 1948; Steinberg et al., 1952; Soffer et al., 1961; Reaven et al., 1974) and increased synthesis and secretion of very-low-density lipoproteins (Stern et al., 1973; Casareto et al., 1974; Bagdade et al., 1976a,b) and in some cases low-density lipoproteins (Bagdade et al., 1976a). Taken together, the above
studies suggest that glucocorticoids may be necessary for maximum expression of insulin-stimulated lipogenesis in the liver and indicate that glucocorticoid excess is associated with increased hepatic lipogenesis.

Our studies confirm and extend these observations. First, as we have previously shown for aminoisobutyrate uptake (Caro & Amatruda, 1982), glucocorticoids in the absence of insulin lead to a profound resistance to the lipogenic effects of subsequently added insulin (Fig. 1). This resistance is due to post-binding mechanisms, since glucocorticoids alone increase insulin binding (Caro & Amatruda, 1982). Thus glucocorticoid-induced insulin resistance can now be generalized to at least two biological responses in the liver. Secondly, the glucocorticoid-induced insulin resistance is reversed after 4 h exposure to insulin in the presence of glucocorticoids (Fig. 4). After this the two hormones act together to potentiate insulin’s stimulation of lipogenesis. This potentiation is also a post-binding enhancement of insulin action, since insulin binding in the presence of insulin plus dexamethasone is the same as that in the presence of dexamethasone alone (Caro & Amatruda, 1982).

Thirdly, the potentiation of insulin action by dexamethasone occurs at glucocorticoid concentrations equivalent to physiological concentrations of cortisol in man (Fig. 3). The onset of the effect is at an equivalent cortisol concentration of 1.5 μg/100 ml and the maximal rate of increase at a cortisol equivalent of 1.5–15 μg/100 ml. This suggests that the effect may be physiologically meaningful. Fourthly, the glucocorticoid effect is observed at physiological concentrations of insulin with an insulin dose–response relationship which is similar to that for insulin alone (Fig. 2). There is, however, a significant rightward shift in the dose–response curve for insulin-stimulated lipogenesis in the presence of dexamethasone (Fig. 2). In conjunction with our previous data, indicating that insulin binding is equally increased in the presence of dexamethasone and dexamethasone plus insulin (Caro & Amatruda, 1982), these data again suggest that alterations in insulin sensitivity in addition to insulin responsiveness can be mediated by post-binding mechanisms (Amatruda et al., 1982).

Finally, our data may have physiological significance for the regulation of normal hepatic lipogenesis and for disease states in man. Since the enhancement of insulin-stimulated lipogenesis is observed at concentrations of glucocorticoids equivalent to physiological values in man, it is likely that the two hormones act together to regulate normal hepatic lipogenesis and perhaps the hyperlipidaemia associated with increased plasma insulin (Olefsky et al., 1974). Also, the heptic steatosis and hyperlipidaemia associated with exogenous or endogenous glucocorticoid excess (Baker et al., 1948; Steinberg et al., 1952; Soffer et al., 1961; Stern et al., 1973; Casaretto et al., 1974; Reaven et al., 1974; Bagdade et al., 1976a,b) can now be explained by an enhancement of insulin-stimulated lipogenesis by glucocorticoids. Since glucocorticoid excess results in increased plasma insulin, the two hormones together would act additively in the liver to enhance lipogenesis. In fact, increased synthesis and secretion of very-low-density lipoproteins have been shown in states of glucocorticoid excess (Bagdade et al., 1976a,b; Stern et al., 1973). This, in conjunction with increased lipoprotein lipase activity (Stern et al., 1973; Pykalisto et al., 1975), might increase adipose-tissue fat stores, leading to enlargement of fat-cells, and subsequent insulin resistance of adipocytes (Czech et al., 1977). This indirect induction of insulin resistance in adipocytes might be additive to other effects of glucocorticoids in these cells to decrease the activity of lipogenic enzymes (Diamant & Shafrir, 1975) and glucose transport (Livingston & Lockwood, 1975). Thus glucocorticoid potentiation of insulin action in the liver in conjunction with increased insulin concentrations may explain many of the observed metabolic alterations associated with hypercortisolism.

Further studies are required to elucidate the mechanism(s) by which glucocorticoids enhance insulin-stimulated hepatic lipogenesis. It is possible, however, that glucocorticoids modulate the synthesis of hepatic lipogenic enzymes and insulin modulates the synthesis and activity of these enzymes.

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References


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