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Dexamethasone Stimulates Insulin Receptor Synthesis in Cultured Rat Hepatocytes*

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The ability of the glucocorticoid dexamethasone to modulate the insulin receptor was examined directly in primary cultures of hepatocytes prepared from adult male rats. Hepatocytes were cultured in a defined medium in the presence and absence of dexamethasone, 0.1 μM. The exposure of hepatocytes to dexamethasone resulted in a time-dependent (steady state by 32 h) increase in insulin binding in both intact hepatocytes and Triton X-100-soluble extracts (total insulin receptor content). The enhanced insulin binding found in soluble extracts of dexamethasone-treated hepatocytes was the result of an increase in insulin receptor number without a change in receptor affinity. In order to assess the mechanism by which dexamethasone "up-regulates" the insulin receptor, the heavy isotope density-shift technique was used to analyze insulin receptor turnover in control and dexamethasone-treated hepatocytes. Hepatocytes were initially cultured for 32 h in standard culture media containing only "light" (^{14}C, ^{12}C, ^{1}H) amino acids. In hepatocytes exposed to dexamethasone, a 417% increase in insulin binding in Triton X-100-soluble extracts was observed. After 32 h, when steady state binding is achieved in dexamethasone-treated cultures, parallel cultures of hepatocytes incubated in the absence and presence of dexamethasone were washed and subsequently cultured in media containing "heavy" amino acids (^{15}N, ^{13}C, ^{2}H). The time-dependent disappearance of light insulin receptor (receptor degradation) and appearance of heavy insulin receptor (receptor synthesis) were monitored using CsCl gradients to resolve the two density species of receptor. At steady state, the rate of receptor synthesis (k_s) was 2.94 and 0.62 fmol of insulin bound h^{-1} in dexamethasone-treated and control hepatocytes, respectively. In contrast to this large increase in the rate of receptor synthesis observed in dexamethasone-treated cells, the first order rate constant for decay (k_d) was the same in dexamethasone-treated (0.074 h^{-1}) and in control (0.077 h^{-1}) hepatocytes. We therefore conclude that glucocorticoid-induced up-regulation of the insulin receptor in the liver is due to stimulation of insulin receptor synthesis.

There is considerable evidence that glucocorticoids can directly influence hormone receptors. For example, adrenal steroids increase the binding of epidermal growth factor to cultured human diploid foreskin (1) and HeLa (2) cells, 1,25-OH-cholecalciferol to bone cells (3), vasopressin to kidney cells (4), and β-adrenergic compounds to human lung cells (5). Several studies have evaluated the effects of glucocorticoids on the insulin receptor, but the results have been contradictory. The *in vivo* administration of glucocorticoids has resulted in an increase (6), decrease (7–11), or no change (7, 11, 12–15) in insulin binding to adipocytes (7, 12, 13), erythrocytes (10, 15), hepatocytes (7, 11), isolated hepatic membranes (8), and monocytes (9, 14, 15). In view of the potential ability of insulin to down-regulate (16) its receptor, it is difficult to assess the direct effect of a specific glucocorticoid when administered *in vivo* since such treatment results in complex alterations in the substrate and hormonal milieu including hyperinsulinemia (8, 11, 14, 15). Direct *in vitro* effects of glucocorticoids on the insulin receptor have been reported, but again no clear pattern has emerged. Glucocorticoids, when added to cultures, have been shown to decrease (17, 18) or exert no effect (13, 19, 20) on insulin binding to adipocytes, increase (21) or decrease (18) insulin binding to cultured fibroblasts, and increase insulin binding to cultured lymphocytes (14, 22, 23). Recently our laboratory has reported that glucocorticoids increase insulin binding to primary cultures of rat hepatocytes and at the same time render these cells resistant to the ability of insulin to down-regulate its receptor (11). Also, we have demonstrated that the *in vivo* effects of glucocorticoids on insulin binding are time-dependent (11).

Because of the complicated *in vivo* alterations in hormonal and substrate milieu, *in vitro* model systems offer an advantage in assessing glucocorticoid action. In view of the central role of the liver in overall metabolism and in insulin action and the numerous specific metabolic effects of glucocorticoids on the liver (11, 24–29), it is important to understand the mechanisms by which glucocorticoids affect the hepatic insulin receptor. It was, therefore, the intent of the present study to examine the mechanism(s) by which glucocorticoids increase the insulin receptor content of rat hepatocytes. Since it has been shown that macromolecular synthesis is often required for glucocorticoid action (30), it would be reasonable to focus on the kinetics of insulin receptor turnover in hepatocytes cultured in the absence and presence of a glucocorti-

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coid. For this purpose, we used the heavy isotope "density-shift" method, first employed in studies on the acetylcholine receptor (31, 32) and more recently to measure the synthesis and degradation of the insulin receptor in differentiating mouse adipocytes (33–35), fibroblasts (21), and chick hepatocytes (36). Our studies show that the exposure of rat hepatocytes to the glucocorticoid dexamethasone results in a time-dependent increase in insulin receptor number without a change in receptor affinity. Furthermore, this increase in the insulin receptor content of cultured rat hepatocytes can be attributed to a dexamethasone-dependent stimulation of receptor synthesis.

EXPERIMENTAL PROCEDURES

Materials—Male Sprague-Dawley rats, approximately 2 months old and fed *ad libitum*, were used for all studies. Carrier-free $Na^{125}I$ was purchased from New England Nuclear, crude collagenase (CLS, 40P162P, 130 units/mg) from Worthington, dexamethasone from Sigma, and ultrapure cesium chloride from Beckman Instruments. An algal-derived heavy amino acid mixture (>95% ^{15}N, ^{13}C, ^{2}H) was kindly supplied by the Division of Research Resources, Los Alamos National Laboratory (Los Alamos, NM) or purchased from Merck, Sharp & Dohme (Montreal, Quebec, Canada). Crystalline porcine insulin was a gift from Dr. Ronald Chance of Lilly.

Preparation and Culture of Rat Hepatocytes—Liver cells were isolated as previously described (37). Isolated hepatocytes were plated on Petri dishes coated with rat tail collagen and maintained at 37 °C in serum-free Waymouth's MB752/1 medium but modified in amino acid composition to contain light (^{14}N, ^{12}C, ^{1}H) amino acids identical to the heavy (^{15}N, ^{13}C, ^{2}H) amino acid content of each selected algal heavy amino acid hydrolysate mixture. In addition, the medium was supplemented with cystine, glutamine, histidine, lysine, and tryptophan. In preliminary experiments, no significant differences in either insulin binding or insulin-dependent lipogenesis were observed in rat hepatocytes cultured in this modified medium compared to cells maintained in Waymouth's MB 752/1 medium containing the standard amino acid composition.

Approximately 3 h after plating, the cells were washed repeatedly at room temperature with Hanks'/HEPES[1] buffer containing albumin (10 mg/ml), and the adherent cells subsequently were cultured in the modified Waymouth's medium. Media changes were made every 16 h.

To study the ability of dexamethasone to modulate insulin binding in cultured rat hepatocytes, the steroid was added at the time of the first media change (3 h) and at each subsequent media change. Aqueous stock solutions were made initially by dissolving the steroid in distilled water followed by filtration (Whatman No. 1 filter paper) and subsequent adjustment to 10 μM (A_{250} = 0.150). From this stock, dilutions were made in culture media.

Insulin Binding in Intact Cultured Rat Hepatocytes—In some studies cell surface insulin binding was assessed directly in monolayers of cultured hepatocytes. ^{125}I-insulin was prepared as described by Cuatrecasas (38). Hepatocytes were washed at room temperature with Hanks'/HEPES buffer containing albumin (10 mg/ml) and glucose (8 mM), and then incubated for 45 min at room temperature in the same buffer. ^{125}I-insulin was added at a trace concentration (0.1 nM). To correct for nonspecific binding, parallel plates containing excess (1 μM) unlabeled insulin were included. The incubation was terminated by repeated washes of the plates with ice-cold phosphate-buffered saline (pH 7.4). The plates were than scraped with a rubber policeman and the cells were transferred to Beckman microcentrifuge tubes and pelleted at 10,000 × *g* for 1 min. Specific binding is expressed as femtomoles of insulin bound/mg of protein.

Insulin Binding in Triton X-100 Extracts from Cultured Rat Hepatocytes—To assess total cellular insulin binding, the plates were first washed free of culture media using phosphate-buffered saline, and then the cells were transferred (with the aid of a rubber policeman) into ice-cold 0.25 M sucrose containing bacitracin (1 mg/ml) to ultracentrifuge tubes. The tubes were centrifuged at 100,000 × *g* at 4 °C for 30 min. The pellet was resuspended in Hanks'/HEPES buffer, pH 7.4, containing 1% (v/v) Triton X-100 and bacitracin (1 mg/ml) but lacking albumin. The suspension was incubated overnight at 4 °C and then centrifuged (100,000 × *g*, 30 min). The supernatant was separated and, after diluting the various extracts with 1% Triton X-100 to achieve identical protein concentrations, stored at −70 °C.

At the time of assay, aliquots of the extracts were further diluted with Hanks'/HEPES buffer containing bacitracin (1 mg/ml) and albumin (10 mg/ml) to yield a final Triton X-100 concentration of 0.1% for the soluble insulin receptor assay. Preliminary experiments showed that insulin binding was linear to soluble protein in a range from 25 to 200 μg of protein. The extracts were incubated overnight at 4 °C with ^{125}I-insulin (0.1 nM) plus increasing concentrations of unlabled insulin. For this purpose, ^{125}I-insulin was additionally purified on a Bio-Gel P30 column. Following incubation, the insulin-receptor complex was separated from unbound insulin by employing the polyethylene glycol precipitation method (39). Scatchard analyses (40) of the binding data were made. Nonspecific binding was taken as the amount of ^{125}I-insulin bound in the presence of 1 μM unlabeled insulin.

Insulin Receptor Turnover in Cultured Rat Hepatocytes—The heavy isotope "density-shift" method of Fambrough (31, 32) as described by Reed and Lane (33) was utilized to determine the rates of insulin receptor synthesis and degradation in rat hepatocytes cultured in the absence and presence of dexamethasone. Hepatocytes were cultured initially for 32 h in media containing only light (^{14}N, ^{12}C, ^{1}H) amino acids. The cells were then washed with media devoid of amino acids and then incubated in media containing an identical composition of heavy (^{15}N, ^{13}C, ^{2}H) amino acids. To prepare the media, a stock solution (5 mg/ml) of heavy amino acids dissolved in phosphate-buffered saline was prepared. The solution was first filtered though an Amicon UM-2 membrane and then passed though a sterile 0.22 μ Millipore filter. The final concentration of heavy amino acids in the medium was 1 mg/ml.

Plates of hepatocytes were allowed to incubate for 0 (32 h after culture in medium containing light amino acids), 3, 5, 7, 11, and 15 h after the switch to media containing heavy amino acids. Following these time intervals, Triton X-100-solubilized receptor extracts were prepared as described above. In order to separate ^{15}N, ^{13}C, ^{2}H-labeled insulin receptors from ^{14}N, ^{12}C, ^{1}H-labeled receptors, 1 mg of soluble extract protein in 0.5 ml of 1% Triton X-100 was mixed with 1.7 ml of CsCl solution (0.568 g/ml in 0.05 M Tris-HCl, pH 7.4, containing 400 units/ml of Trasylol). The mixture was overlaid with paraffin oil and centrifuged in a Beckman SW Ti-60 rotor at 50,000 rpm (305,000 × *g*) for 18 h at 4 °C. The tubes were then pierced, and 50-μl fractions were collected. Each fraction was diluted with Hanks'/HEPES buffer containing bacitracin (1 mg/ml) and albumin (10 mg/ml) and then divided into two portions. One portion was incubated with 0.85 nM ^{125}I-insulin alone and the second portion was incubated with ^{125}I-insulin plus 1 μM unlabeled insulin. The soluble insulin receptor content was then assessed as described above.

In order to calibrate the CsCl gradients, a Bausch and Lomb refractometer was employed. The refractive index of representative fractions was measured in parallel centrifugations.

Protein Assay—The protein content of soluble extracts was measured by the method of Lowry *et al.* (41) as modified by Miller (42). The protein content of ^{125}I-insulin pellets was measured by digesting the pellet initially in 2 N NaOH at 60 °C.

RESULTS

Time Course of Up-regulation of the Insulin Receptor by Dexamethasone in Cultured Rat Hepatocytes—The ability of the synthetic glucocorticoid dexamethasone to alter insulin binding in rat hepatocytes was examined in cells cultured in the absence and presence of 0.1 μM dexamethasone. Trace (0.1 nM) insulin binding was measured in intact hepatocytes as well as in Triton X-100-soluble extracts. The latter represents total cellular insulin binding. Cultures of rat hepatocytes, when exposed either overnight (12 to 16 h) or for longer periods (22 to 36 h) to dexamethasone, had higher insulin binding (45 ± 20% increase at 12 to 16 h and 178 ± 62% increase at 22 to 36 h) than hepatocytes cultured in medium alone. The increased insulin binding was not the result of a dexamethasone-dependent effect on insulin degradation activity in the media since the amount of trichloroacetic acid-soluble radioactivity was similar (~1.5%) in control and dexamethasone-treated hepatocytes incubated with ^{125}I-insu-

[1] The abbreviation used is: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

lin. This enhanced insulin binding found in intact hepatocytes was also evident in soluble extracts of cultured hepatocytes (52 ± 20% increase at 12 to 16 h and 113 ± 40% increase at 20 to 36 h). The exposure of rat hepatocytes to dexamethasone consistently resulted in time-dependent increases in insulin binding with a steady state achieved by 32 h, although the per cent increase was highly variable between different preparations of hepatocytes.

The ability of dexamethasone to increase insulin binding in both intact and Triton X-100-solubilized hepatocytes was found to be dose-dependent. A stimulation of insulin binding could be observed at 0.1 nM dexamethasone (28% maximum) with a near maximal (85%) response at 10 nM dexamethasone and a maximal response at 0.1 μM dexamethasone.

Scatchard Analyses of the Dexamethasone-induced increase in Insulin Binding to Soluble Extracts—The increase in insulin binding consequent to treatment with dexamethasone was analyzed by the method of Scatchard (40). As shown in Fig. 1, the exposure of hepatocytes to dexamethasone resulted in increases in the number of both high and low affinity binding sites. No apparent alteration in the affinity of the insulin-receptor interaction was evident. An insulin concentration of 0.85 nM (Fig. 1, *arrow*) was used in subsequent experiments assessing insulin receptor turnover.

Insulin Receptor Turnover in Control and Dexamethasone-treated Rat Hepatocytes—The exposure of cultured rat hepatocytes to dexamethasone resulted in an increase in both surface binding (intact hepatocytes) and in the total cellular receptor content (soluble extracts). To determine whether this increase in insulin binding was the result of a dexamethasone-dependent increase in insulin receptor synthesis or a decrease in the rate of receptor degradation, or both, the heavy isotope density-shift technique was used as described under "Experimental Procedures." Fig. 2 illustrates the time course profile of the loss of light insulin receptor (degradation) and appearance of heavy receptor (synthesis) in soluble extracts from primary cultures of hepatocytes incubated in the absence and presence of dexamethasone. At time zero, a single receptor species was found as a distinct peak with the peak height fraction localized in the same position (fraction 27, refractive index = 1.3620 η^{D25}) in gradients from control (control gradient) or dexamethasone-treated (dexamethasone gradient) hepatocytes. Subsequently, a time-dependent reduction in the light receptor peak in control and dexamethasone gradients was found as well as an appearance of newly synthesized heavy receptor. The total binding on the control gradient was 9.20 fmol of insulin bound and on the dexamethasone gradient, 47.60 fmol of insulin bound. Thus, there was a 417% increase in binding due to dexamethasone treatment.

In gradients containing both heavy and light receptor (for control gradients, first evident at 5 h and for dexamethasone gradients 3 h) the heavy receptor peak height fraction from control and dexamethasone gradients was located 6 to 8 fractions from the corresponding light receptor peak height

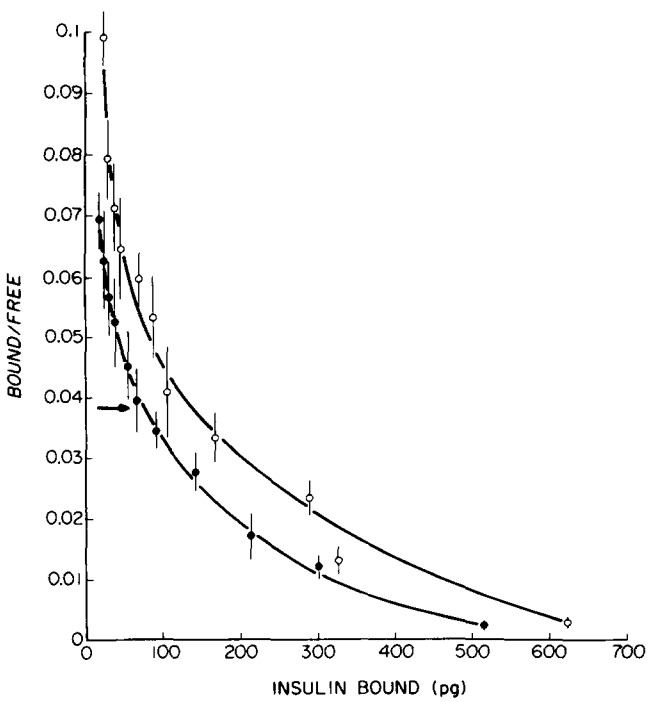

FIG. 1. **Scatchard analyses of ^{125}I-insulin binding to soluble insulin receptors from primary cultures of rat hepatocytes.** The conditions of cell isolation and plating are described under "Experimental Procedures." Rat hepatocytes were cultured for 24 h in the absence (●) and presence (○) of dexamethasone (0.1 μM). The monolayers were washed and ^{125}I-insulin binding was determined in Triton X-100-soluble extracts as described under "Experimental Procedure." Each point represents the mean ± S.E. of three separate experiments. In each experiment, a soluble extract was prepared from 6 to 10 replicate plates of hepatocytes. The *arrow* indicates the bound/free ratios in the presence of a total insulin concentration of 0.85 nM, the concentration used in the heavy isotope density shift experiments.

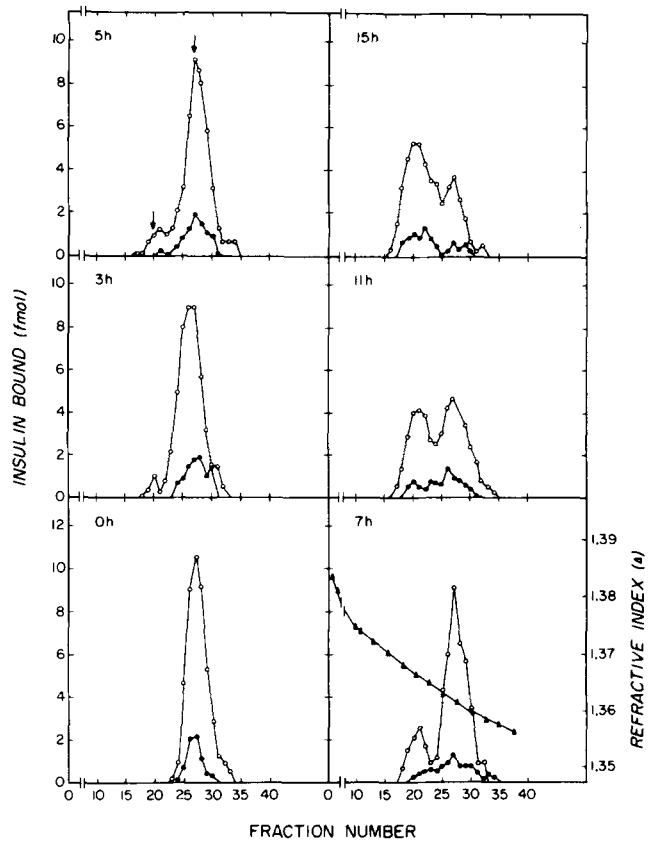

FIG. 2. **Time course profiles of the isopycnic binding of soluble insulin receptors from primary cultures of rat hepatocytes.** Rat hepatocytes were cultured in the absence (●) and presence (○) of dexamethasone (0.1 μM) in media containing only light (^{14}N, ^{12}C, ^{1}H) amino acids. After 32 h (referred to as 0 h), the cells were switched to media containing an identical composition of heavy (^{15}N, ^{13}C, ^{2}H) amino acids and incubated for the times indicated. Triton X-100-soluble extracts were prepared and CsCl density gradients were employed to resolve light and heavy insulin receptor species as described under "Experimental Procedures." The *arrows* indicate the approximate positions of the light and heavy peak height fractions. The results are from a representative experiment of three separate experiments.

fraction. In gradients containing both light and heavy insulin receptor, there was a common low binding fraction or nadir between the two well separated peak height fractions. This fraction represented a zone of overlap between the two insulin receptor species. To calculate the total light receptor peak area, the insulin binding measured in all gradient fractions less dense than this overlapping zone plus one-half of the common fractions were totaled. The heavy receptor peak area was taken to include one-half of the common fraction and the binding in the more dense fractions. In both control and dexamethasone gradients, insulin receptor synthesis showed a time-dependent linear increase beginning about 4 h after the switch from light to heavy amino acids (Fig. 2).

Kinetic curves of insulin receptor synthesis and receptor degradation were constructed using the data from the time course profile and are shown in Figs. 3 and 4, respectively. From these curves, the rate of receptor synthesis (k_s) in control gradients, is 0.62 fmol bound h^{-1}, whereas the rate of insulin receptor synthesis in hepatocytes cultured in the presence of dexamethasone is 2.94 fmol h^{-1}. Thus, exposure to a glucocorticoid resulted in a dramatic increase (approximately 4.8-fold) in steady state receptor synthesis.

A semilogarithmic plot of receptor decay is shown in Fig. 4. After a lag of about 3 h, there was a time-dependent loss of the light receptor peak in both control and dexamethasone gradients. However, no major differences were found in the half-life of the insulin receptor (Fig. 4, *arrows*) from either

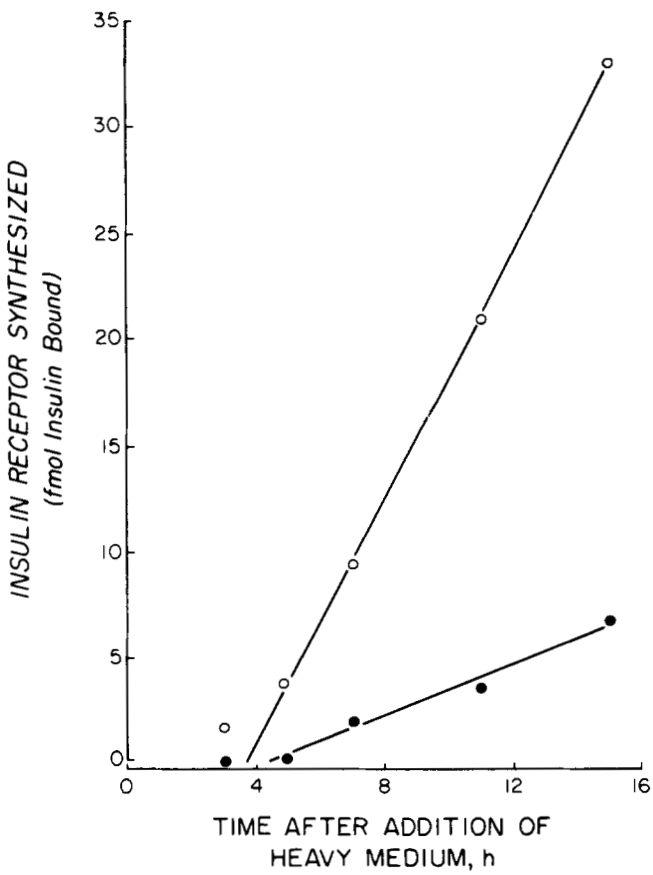

FIG. 3. **Insulin receptor synthesis in cultured rat hepatocytes.** The amount of heavy insulin receptor present at each time point was calculated from the relative peaks areas shown in Fig. 2 for rat hepatocytes cultured in the absence (●) or presence (○) of dexamethasone (0.1 μM). From these curves, the rates of insulin receptor synthesis (k_s) in control and dexamethasone-treated hepatocytes were 0.62 and 2.94 fmol of insulin bound h^{-1}, respectively.

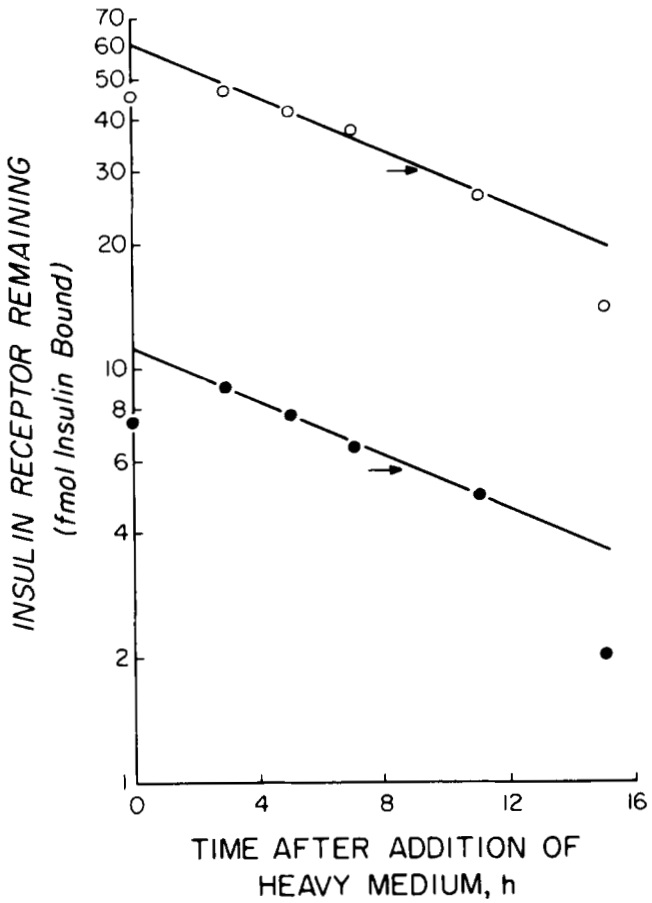

FIG. 4. **Insulin receptor decay in cultured rat hepatocytes.** The amount of light insulin receptor remaining at each time point was calculated from the relative peak areas shown in Fig. 2 for rat hepatocytes cultured in the absence (●) and presence (○) of dexamethasone (0.1 μM). From these semilogarithmic curves, the first order decay rate constants (k_d) in control and dexamethasone-treated hepatocytes were 0.077 and 0.074 h^{-1}, respectively.

TABLE I

Kinetic constants of insulin binding in control and dexamethasone-treated hepatocytes

Dexamethasone (0.1 μM)	k_s[a]	$t_{1/2}$[b]	k_d[c]	Insulin binding (R_t)	
				Predicted[d]	Measured
		h	h^{-1}	*fmol bound*	
−	0.62	9.0	0.077	8.05	9.20
+	2.94	9.4	0.074	39.73	47.60

[a] Expressed as fmol of bound insulin synthesized h^{-1}, determined from the slope of the line shown in Fig. 3.
[b] Receptor half-life determined from the slope of the line shown in Fig. 4.
[c] First order decay constant where $kd = 0.693/t_{1/2}$.
[d] Calculated from $R_t = ks/kd$.

control ($t_{1/2}$ = 9.0 h) or dexamethasone-treated ($t_{1/2}$ = 9.4 h) hepatocytes. The first order rate constants (k_d) for insulin receptor decay were calculated to be 0.077 h and 0.074 h^{-1} in control and dexamethasone-treated hepatocytes, respectively. Table I summarizes the data obtained from Figs. 3 and 4. It is readily apparent that the predicted level of total insulin binding (R_t) calculated from the rate of receptor synthesis (k_s) and the first order rate constant (k_d, where $R_t = k_s/k_d$) approximates the level of binding activity experimentally determined in the control and dexamethasone gradients.

DISCUSSION

Glucocorticoid administration has been shown to modulate the insulin receptor in a variety of cells (6–11). However, the results of these studies have varied, depending on the glucocorticoid under study, the time course of administration, and the cell type used to assess insulin binding. In view of the diverse effects of glucocorticoids on many metabolic processes (11, 13, 15, 18–20, 24–30), this variation should not be unexpected. However, any attempt to utilize *in vivo* models to assess the ability of a glucocorticoid to modulate the insulin receptor is fraught with other pertubations associated with administration of the glucorticoid such as increases in plasma insulin (8, 11, 14, 15), glucagon (43), and ketone bodies (6), all of which are either demonstrated (11, 16, 44–46) or potential modulators of the insulin receptor. Employing *in vitro* model systems, various investigators have studied the direct effect of glucocorticoids on the insulin receptor (11, 17–23). In some of these studies (11, 14, 21–23) positive or up-regulation of the insulin receptor by a glucocorticoid was found. Fantus *et al.* (14) exposed cultured human (IM9) lymphocytes to hydrocortisone for 18 h and found a dose-dependent increase (2-fold) in insulin binding. Scatchard analysis showed the increase in binding to be the result of an affinity change. Knopf *et al.* (22) also reported a dose-dependent increase in insulin binding in cultured lymphocytes exposed to hydrocortisone, but attributed the increase solely to an increased number of binding sites without any alteration in affinity.

During the course of the present investigation, two studies (21, 23) were published which examined the mechanism by which glucocorticoids up-regulate the insulin receptor in cultured cells. Knutson *et al.* (21) found that the exposure of 3T3 mouse fibroblasts to dexamethasone resulted in a time-dependent increase in insulin binding activity as assessed in both intact cells and in Triton X-100-solubilized extracts. Scatchard analysis of the insulin binding data revealed no alterations in the affinity of the insulin receptor for insulin as a result of exposure to dexamethasone. Utilizing the heavy isotope density-shift technique, these authors found that dexamethasone decreased the first order rate constant for receptor decay approximately 2-fold. The $t_{1/2}$ of the insulin receptor was 10.2 h in control fibroblasts and increased to 18 h in the dexamethasone-treated cells. In contrast, Fantus *et al.* (23) observed a dexamethasone-dependent stimulation of insulin receptor synthesis in cultured human IM9 lymphocytes. In their study, receptor degradation was assessed by precipitating solubilized ^{125}I-labeled cellular extracts with insulin receptor antibodies at specific time intervals. Using this approach, these authors demonstrated an actual enhancement of insulin receptor degradation with glucocorticoid treatment. By inference, they concluded that *de novo* synthesis of insulin receptor must account for the appearance of additional insulin binding sites. The results from the present study of dexamethasone-dependent up-regulation in rat hepatocytes demonstrate that the increase in insulin binding can be entirely attributed to a stimulation of insulin receptor synthesis. No significant alterations in the first order rate constant for insulin decay were found. In this regard it is of interest that the kinetics of insulin receptor decay is similar in all cell types examined to date. The $t_{1/2}$ of 9 h found for control hepatocytes in the present study is similar to the values (9–11 h) reported in other studies of insulin receptor degradation in untreated chick hepatocytes (36) and mouse adipocytes (33–35) and fibroblasts (21) employing the heavy isotope density-shift technique and in studies using cultured human lymphocytes (23, 47) in which insulin receptor decay was assessed with insulin receptor antibodies.

Although our results suggest that dexamethasone increased the number of insulin receptors by stimulating receptor synthesis in rat hepatocytes, other interpretations should be considered. For example, it could be suggested that the observed increase in insulin binding may be the result of a dexamethasone-dependent unmasking of cryptic insulin receptor sites. However, since we observed increases in insulin binding in both Triton X-100-soluble extracts as well as in intact hepatocytes, this possibility seems unlikely. Since we (11) and others (48) have shown that dexamethasone does not affect the rate of amino acid uptake in cultured rat hepatocytes, it is also unlikely that changes in amino acid pools could account for our results. Another possibility is that the observed dexamethasone-dependent up-regulation of the insulin receptor may represent a general ability of dexamethasone to maintain viable rat hepatocytes cultured in a serum-free medium. In this regard, in the dexamethasone-treated hepatocytes in which we observed a 4.8-fold increase in insulin binding compared to untreated cells, only a 40% enhancement of protein synthesis was found in parallel cultures treated identically. Therefore, it would appear that dexamethasone is acting on specific metabolic parameters.

In the present study, up-regulation of the insulin receptor could be observed in hepatocytes exposed to 0.1 nM dexamethasone, a concentration equal in potency to 0.28 μg/ml of corticosterone, with near maximal response found at 10 nM dexamethasone. This concentration of glucocorticoid, which is equivalent to 28 μg of corticosterone/100 ml, is well within the physiological range in nonstressed rats.

What is the function of these newly synthesized insulin receptor sites in rat hepatocytes? Considerable *in vitro* evidence exists which demonstrates that glucocorticoids can potentiate insulin action in the liver (25–29, 49). For example, the preincubation of cultured hepatocytes with glucocorticoids results in a permissive effect on the ability of insulin to increase the activities of glycogen synthetase (27) and glucokinase (26, 28). Prolonged incubation with both insulin and dexamethasone results in marked potentiation of insulin-stimulated lipogenesis (49). It would be tempting to speculate that the permissive effect of glucocorticoids to potentiate insulin action in liver is through a positive modulation of the insulin receptor. However, we (11) have previously listed many studies from the literature in which insulin binding and response were not coupled and which demonstrate that events beyond the binding of insulin to its receptor may regulate responsiveness in insulin target cells. Such is the case in our recent studies showing that the pretreatment of hepatocytes with dexamethasone for 16 h in the absence of insulin results in increased insulin binding but renders the cell resistant to insulin-dependent aminoisobutyric acid uptake (11) and lipogenesis (49). In contrast, with pretreatment with both insulin and dexamethasone for 16 h, insulin binding is similarly increased with profound enhancement of insulin action (49). Thus, the same alteration in insulin binding is associated with both enhanced and impaired insulin action depending on the biological response evaluated (46) or the conditions of incubation (49). Such studies reinforce the importance of postinsulin binding events. It will be of importance to examine additional parameters of insulin action in cultured hepatocytes including glycogen synthesis in other to identify which, if any, biological response(s) to insulin can be coupled to the dexamethasone-induced increase in insulin receptor levels. These studies are currently under investigation.

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REFERENCES

1. Baker, J. B., Barsh, G. S., Carney, D. H., and Cunningham, D. D. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75,** 1882–1886
2. Wu, R., Wolfe, R. A., and Sato, G. H. (1981) *J. Cell Physiol.* **108,** 83–90
3. Manolagers, S. C., and Anderson, D. C. (1979) *Nature (Lond.)* **277,** 314–315
4. Rajerison, R., Marchetti, J., Roy, C., Bockaert, J., and Jard, S. (1974) *J. Biol. Chem.* **249,** 6390–6400
5. Fraser, G. M., and Venter, J. C. (1979) *Fed. Proc.* **38,** 701A
6. Beck-Nielsen, H., De Pirro, R., and Pederson, O. (1980) *J. Clin. Endocrinol. Metab.* **50,** 1–4
7. Olefsky, J., Johnson, J., Liu, F., Jen, P., and Reaven, G. (1975) *Metab. Clin. Exp.* **24,** 517–527
8. Kahn, R., Goldfine, I., Neville, D., and DeMeyts, P. (1978) *Endocrinology* **103,** 1054–1066
9. De Pirro, R., Bertoli, A., Fusco, A., Testa, I., Greco, A. V., and Lauro, R. (1980) *J. Clin. Endocrinol. Metab.* **51,** 503–507
10. Yasuda, K., and Kitabchi, A. (1980) *Diabetes* **29,** 811–814
11. Caro, J. F., and Amatruda, J. M. (1982) *J. Clin. Invest.* **69,** 866–875
12. Bennet G. V., and Cuatrecasas, P. (1972) *Science (Wash. D. C.)* **276,** 805–806
13. DePirro, R., Green, A., Kao, M., and Olefsky, J. (1981) *Diabetologia* **21,** 149–153
14. Fantus, I. G., Ryan, J., Hizuka, N., and Gorden, P. (1981) *J. Clin. Endocrinol. Metab.* **52,** 953–960
15. Rizza, R. A., Mandarino, L. J., and Gerich, J. E. (1982) *J. Clin. Endocrinol. Metab.* **54,** 131–138
16. Gavin, J. R., III, Roth, J., Neville, D. M., Jr., DeMeyts, P., and Buell, D. N. (1974) *Proc. Natl Acad. Sci. U. S. A.* **71,** 84–88
17. Cigolini, M., and Smith, U. (1979) *Metab. Clin. Exp.* **28,** 502–520
18. Grunfeld, C., Baird, K., Van Obberghen, E., and Kahn, C. R. (1981) *Endocrinology* **109,** 1723–1730
19. Olefsky, J. M. (1975) *J. Clin. Invest.* **56,** 1499–1508
20. Malchoff, D. M., Maloff, B. L., Livingston, J. N., and Lockwood, D. H. (1982) *Endocrinology* **110,** 2081–2087
21. Knutson, V. P., Ronnett, G. V., and Lane, M. D. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 2822–2826
22. Knopf, R. F., Torretti, B. A., and Hossler, P. A. (1978) in *Proceedings of the 60th Annual Meeting of the Endocrine Society, Miami,* p. 512A
23. Fantus I. G., Saviolakis, G. A., Hedo, J. A., and Gorden, P. (1982) *J. Biol. Chem.* **257,** 8277–8283
24. Reed, G. B., and Grisham, J. W. (1975) *Lab. Invest.* **33,** 298–304
25. Plas, C., and Nunez, J. (1976) *J. Biol. Chem.* **251,** 1431–1437
26. Shudt, C. (1979) *Eur. J. Biochem.* **98,** 77–82
27. Shudt, C. (1979) *Eur. J. Biochem.* **97,** 155–160
28. Katz, N. A., Nauck, M. A., and Wilson, P. T. (1979) *Biochem. Biophys. Res. Commun.* **88,** 23–29
29. Lin, R. C., Snodgrass, P. J., and Rabier, D. (1982) *J. Biol. Chem.* **257,** 5061–5067
30. Baxter, J. D., and Forsham, P. H. (1972) *Am. J. Med.* **53,** 573–589
31. Devreotes, P. N., Gardner, J. M., and Fambrough, D. M. (1977) *Cell* **10,** 365–373
32. Gardner, J. M., and Fambrough, D. M. (1979) *Cell* **16,** 661–674
33. Reed, B. C., and Lane, M. D. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 285–289
34. Reed, B. C., Ronnett, G. V., Clements, P. R., and Lane, M. D. (1981) *J. Biol Chem.* **256,** 3917–3925
35. Ronnett, G. V., Knutson, V. P., and Lane, M. D. (1982) *J. Biol. Chem.* **257,** 4285–4291
36. Krupp, M., and Lane, M. D. (1981) *J. Biol. Chem.* **256,** 1689–1694
37. Cech, J. M., Freeman, R. B., Jr., Caro, J. F., and Amatruda, J. M. (1980) *Biochem. J.* **188,** 839–845
38. Cuatrecasas, P. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68,** 1264–1268
39. Cuatrecasas, P. (1972) *Proc. Natl. Acad. Sci. U. S. A.* **69,** 318–322
40. Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.* **51,** 660–672
41. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275
42. Miller, G. L. (1959) *Anal.Chem.* **31,** 964
43. Marco, J., Calle, C., Roman, D., Diaz-Fierros, M., Vilanueva, M., and Valverde, I. (1973) *N. Engl. J. Med.* **288,** 128–131
44. Misbin, R. I., Pulkkinen, A. J., Lofton, S. A., and Merimee, T. J. (1978) *Diabetes* **27,** 539–542
45. Kosmakos, F. C., and Roth, J. (1980) *J. Biol. Chem.* **255,** 9860–9869
46. Amatruda, J. M., Newmeyer, H. W., and Chang, C. L. (1982) *Diabetes* **31,** 145–148
47. Kasuga, M., Kahn, C. R., Hedo, J. A., Van Obberghen E., and Yamada, K. M. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 6917–6921
48. Kletzien, R. F., Pariza, M. W., Becker, J. E., and Potter, V. R. (1976) *J. Cell. Physiol.* **89,** 641–646
49. Amatruda, J. M., Danahy, S. A., and Chang, C. L. (1983) *Biochem. J.* **212,** 135–141

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