

Glucocorticoid Regulation of ACTH Sensitivity of Adenyl Cyclase in Rat Fat Cell Membranes

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Abstract. Plasma membrane sacs of isolated rat fat cells (ghosts) possess an adenyl cyclase system, which is activated by lipolytic hormones of disparate molecular structure, including adrenocorticotropin (ACTH), glucagon, and epinephrine. Previous studies indicated that distinctive selectivity units for individual hormones are coupled to the same unit of adenyl cyclase in the fat cell membrane. The present study has shown that ghost cyclase from adrenalectomized and hypophysectomized rats exhibits a striking reduction in response to ACTH, the stimulatory effects of epinephrine, glucagon, or fluoride being unchanged. Pretreatment of adrenalectomized, hypophysectomized, sham operated, or intact rats with the synthetic glucocorticoid, dexamethasone, selectively increased the ACTH response in ghost cyclase preparations. Cortisol, like dexamethasone, increased the ACTH response in ghosts from adrenalectomized rats; 11-deoxycorticosterone was ineffective. The dexamethasone effect to enhance the ACTH response is blocked by actinomycin D or cycloheximide.

The present results show that stimulation of rat fat cell adenyl cyclase by ACTH involves a distinctive molecular entity, which can be clearly differentiated from adenyl cyclase in the membrane as well as from the selectivity sites for epinephrine and glucagon. The data indicate that the biosynthesis of the component required for ACTH stimulation of ghost cyclase—either an ACTH selectivity unit or specific coupling factor—is induced by glucocorticoids at the level of gene regulation.

A number of peptide and protein hormones as well as catecholamines evoke specific responses in mammalian target tissues *via* the adenyl cyclase-3',5'-AMP system.¹ In many hormone-responsive tissues, the cyclase is highly selective and is activated by a single hormone (ACTH in adrenal cortex,² thyroid stimulating hormone in thyroid,³ epinephrine in avian erythrocytes,⁴ luteinizing hormone in corpus luteum⁵). The cyclase of rat fat cells is distinctive in that it is stimulated by at least six lipolytic hormones of disparate molecular structure: ACTH, glucagon, epinephrine,⁶⁻⁸ thyroid stimulating hormone,⁶ luteinizing hormone,⁶ and secretin.⁹

The nature of hormone selectivity in adenyl cyclase systems has been most extensively studied in rat fat cell membrane preparations. A variety of studies^{6,7,9} provide strong evidence for the view that hormone selectivity in the ghost cyclase system depends upon distinctive molecular units (designated as hormonal

discriminators⁷) all coupled to a single unit of adenylyl cyclase in the membrane. The present work which shows that glucocorticoids selectively induce a distinctive molecular entity required for ACTH stimulation of ghost cyclase provides additional support for this view.

Methods and Materials. Male Sprague-Dawley rats, weighing 120–160 g, maintained at $22 \pm 0.5^\circ\text{C}$, permitted free access to Purina Laboratory chow diet, were used in these experiments. In each experiment, one group of rats was adrenalectomized (or hypophysectomized); another group subjected to “sham” operation served as control. The adrenalectomized and hypophysectomized rats received supplemental horsemeat in their diet to stimulate food intake. The adrenalectomized rats had free access to 0.9% NaCl solution for drinking; all other groups had water. Fat cell ghosts were prepared from pooled epididymal fat cells of various groups of rats, using the technique of Rodbell.¹⁰ The ghosts were suspended in 1 mM KHCO_3 containing 0.1% bovine serum albumin and assayed either soon after preparation or after rapid freezing (in small batches) and storage at -60°C . Adenylyl cyclase activity was assayed using conditions previously described.^{7,11} ACTH, glucagon, epinephrine, and NaF dose-response curves were obtained on samples of treated and control preparations in the same assay. The results were expressed in picomoles of 3',5'-AMP formed per milligram fat cell ghost protein per minute. The basal cyclase activities of ghost preparations varied within similar groups from experiment to experiment; accordingly, the response of ghost cyclase to various stimulatory agents was evaluated in terms of percentage increase over the basal value.

β^{1-24} -ACTH was a gift of Dr. W. Rittl, Ciba, Basel, Switzerland; synthetic glucagon was a gift of Dr. E. Jaeger, Max-Planck Institute, Munich, Germany; actinomycin D was a gift of Merck, Sharp and Dohme, New Jersey, USA; L-epinephrine, dexamethasone, and cycloheximide were purchased from Sigma; cortisol and 11-deoxycorticosterone were from Calbiochem.

Results. Ghost cyclase obtained from intact normal or sham-operated rats is stimulated 210% (range 100–300) over basal values by maximal concentrations of ACTH. Fat cell ghosts obtained from rats adrenalectomized for 6–24 days consistently exhibit decreased sensitivity to ACTH, relative to ghosts from sham-operated control rats, as expressed in terms of *magnitude* of cyclase activation produced by maximal concentrations of ACTH; the *affinity*

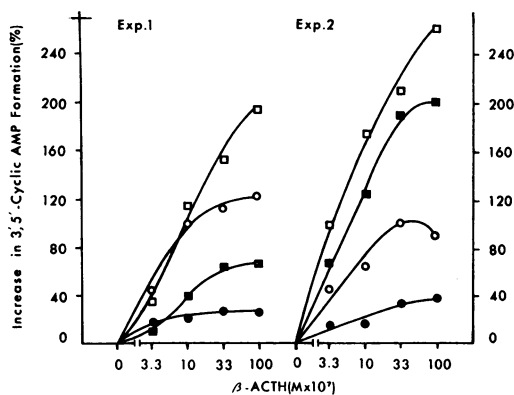


Fig. 1.—Effect of dexamethasone treatment of sham-operated and adrenalectomized rats on the response of fat cell ghost adenylyl cyclase to ACTH. —○—, sham-operated, placebo treated; —□—, sham-operated, dexamethasone treated; —●—, adrenalectomized, placebo treated; —■—, adrenalectomized, dexamethasone-treated rats. Each point is the mean of two determinations which agreed very closely.

Expt. 1: Ghosts were prepared from rats 6 days after surgery. Dexamethasone (0.5 mg/100 g body weight) was divided in three separate injections (at 0, 2, and 4 hr). Animals were sacrificed and fat pads dissected 8 hr after the first injection.

Expt. 2: Ghosts were prepared from rats 12 days after surgery. Dexamethasone (0.5 mg/100 g body weight) was divided in five separate injections (at 0, 10, 24, 34, and 48 hr). Animals were sacrificed and fat pads dissected 52 hr after the first injection.

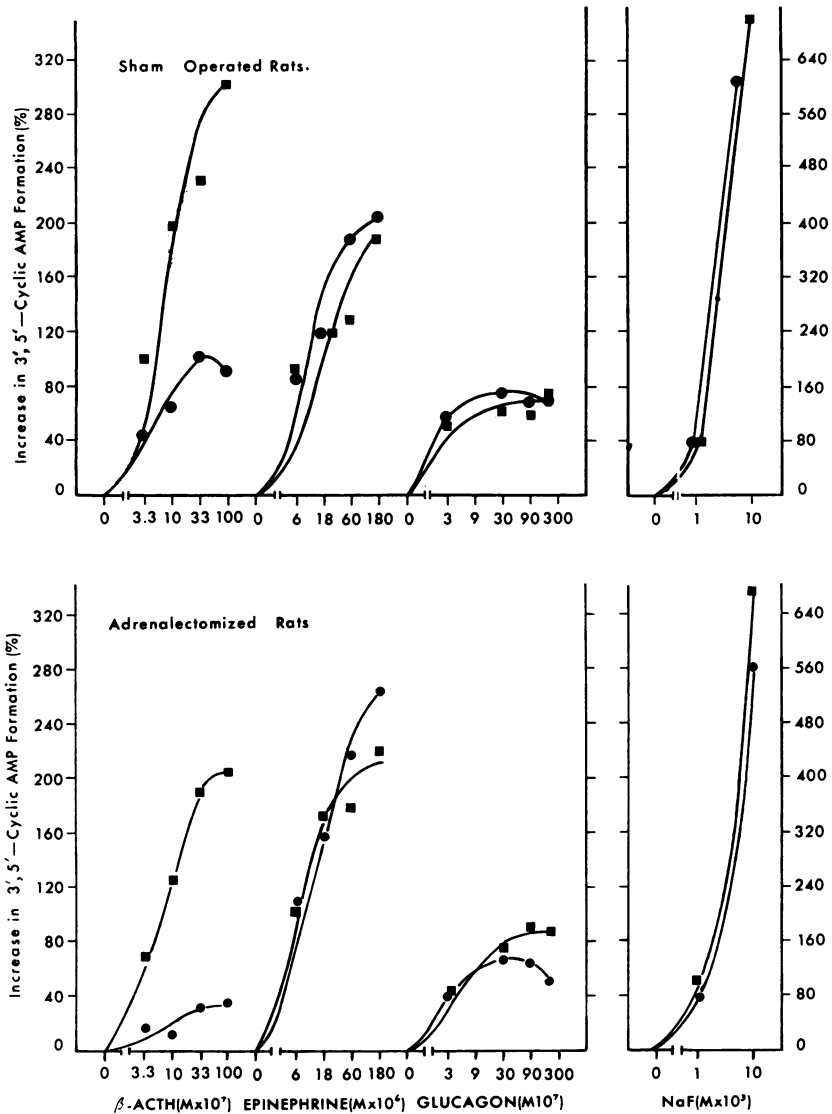


FIG. 2.—Effect of dexamethasone treatment of sham-operated and adrenalectomized rats on the response of ghost cyclase to ACTH, epinephrine, glucagon, and NaF. —●—, control, placebo treated groups; —■—, dexamethasone-treated groups. Dexamethasone (total dose 0.5 mg/100 g body weight) was injected intraperitoneally in five separate injections over 52 hr. Each point is the mean of two determinations which agreed very closely.

parameter (concentration of ACTH required for half-maximal stimulation) was not influenced by adrenalectomy. In six experiments, the magnitude of ACTH response with ghosts from adrenalectomized rats was only 20% (range 12–30) of that achieved in the control; the stimulatory effects of epinephrine, glucagon, and NaF upon preparations of ghost cyclase from adrenalectomized rats was con-

sistently found to be unchanged. ACTH sensitivity was decreased to the same extent, whether the rats were adrenalectomized for 6 or 24 days.¹²

The selective decrease in the response to ACTH exhibited by ghost cyclase after adrenalectomy was restored, or even increased to supernormal levels, by treatment of adrenalectomized rats with dexamethasone; the extent of increase was found to depend upon the time period of dexamethasone treatment. Figure 1 shows typical experiments where 0.5 mg dexamethasone/100 g body weight was administered in multiple doses to adrenalectomized rats over 8 and 52 hr. Dexamethasone administered over 52 hr produced supernormal sensitivity to ACTH; the same total dose of dexamethasone administered over 8 hr increased ACTH response significantly but not to the normal level. Prolonged treatment with dexamethasone increased ACTH sensitivity to supernormal levels not only in adrenalectomized rats but in sham-operated rats as well. In both groups the dexamethasone effect is specific for ACTH, the response to epinephrine, glucagon, and NaF being unchanged (Fig. 2). Treatment of adrenalectomized rats with cortisol enhanced the response to ACTH, but cortisol was less effective than dexamethasone.¹³ 11-deoxycorticosterone was found to be without any significant effect. The effect of adrenalectomy on the response to ACTH thus appears to be caused by a glucocorticoid deficiency.

Ghost cyclase from rats hypophysectomized for 6 days to 3 months, like that from adrenalectomized rats, exhibited a selective decrease in response to ACTH; the response to epinephrine and NaF was essentially similar to that obtained with cyclase from sham-operated controls. In the early stage after hypophysectomy, dexamethasone (administered over 60 hr) prevented the decline in ACTH sensitivity observed after pituitary removal, but did not give rise to supernormal ACTH sensitivity; 18 days after hypophysectomy, dexamethasone treatment still enhanced the stimulatory effect of ACTH but the effect was less pronounced (Fig. 3).

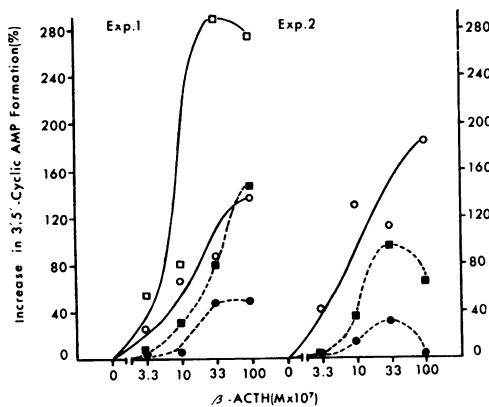


FIG. 3.—Effect of dexamethasone treatment of hypophysectomized and sham-operated rats on the response of fat cell ghost adenyl cyclase to ACTH. Each point is the mean of two determinations which agreed very closely.

Expt. 1: Ghosts were prepared from rats 6 days after surgery. —○—, sham-operated, placebo treated; —□—, sham-operated, dexamethasone treated; —●—, hypophysectomized, placebo treated; and —■—, hypophysectomized dexamethasone-treated rats. Dexamethasone (total dose 0.5 mg/100 g body weight) was injected intraperitoneally in 5 separate injections (at 0, 10, 24, 34, and 48 hr). The animals were sacrificed and the fat pads were removed 60 hr after the first injection.

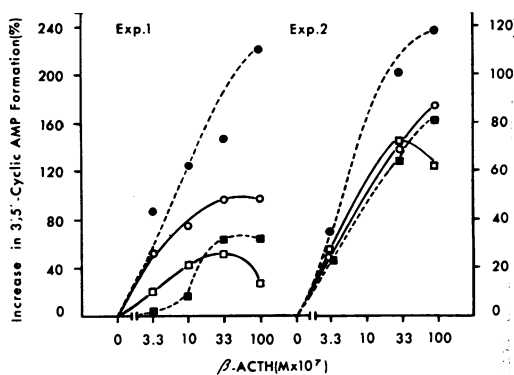
Expt. 2: Ghosts were prepared from rats 18 days after surgery. Experimental conditions and the symbols used are the same as in Expt. 1.

Dexamethasone treatment thus leads to a selective increase in the response to ACTH of ghost adenylyl cyclase from adrenalectomized, hypophysectomized, sham-operated, and intact normal rats. The effect of dexamethasone to augment ACTH stimulation was blocked by simultaneous administration of either actinomycin D, which is known to inhibit DNA-dependent RNA synthesis,¹⁴ or cycloheximide, which is known to inhibit protein synthesis.¹⁵ Figure 4 shows typical

FIG. 4.—Inhibition of the dexamethasone effect by simultaneous administration of actinomycin D or cycloheximide.

Expt. 1: Groups of intact rats were treated with placebo injections —○—, dexamethasone (total dose 0.4 mg/100 g body weight) —●—, actinomycin D (total dose 40 μ g/100 g body weight) —□—, and dexamethasone plus actinomycin D simultaneously —■—, divided in four separate injections (at 0, 10, 24, and 34 hr). The fat pads were dissected 48 hr after the first injection.

Expt. 2: Groups of sham-operated rats were treated with placebo injections —○—, dexamethasone (total dose 0.3 mg/100 g body weight) —●—, cycloheximide (total dose 0.3 mg/100 g body weight) —□—, and dexamethasone plus cycloheximide simultaneously —■—, divided in three separate injections (at 0, 12, and 24 hr). The fat pads were dissected 40 hr after the first injection.



experiments in intact and sham-operated rats where the effect of dexamethasone to enhance the stimulatory effect of ACTH on ghost adenylyl cyclase was blocked by actinomycin D or cycloheximide. Neither actinomycin D or cycloheximide influenced the response of cyclase to epinephrine or NaF. Similar results were obtained with adrenalectomized rats.¹⁶

Discussion. Previous studies have indicated that distinctive selectivity sites for individual lipolytic hormones in the rat fat cell membrane are coupled to the same unit of adenylyl cyclase.⁶⁻⁸ Distinctive selectivity sites for ACTH, epinephrine, and glucagon were differentiated as follows: the selective site for ACTH had a specific requirement for Ca^{2+} and was selectively inhibited by an ACTH analog;⁶ the selective site for epinephrine was differentiated with β -adrenergic blocking agents;^{6,7} the glucagon site was differentiated by preincubating intact fat cells with trypsin.⁹ Ghosts prepared from trypsin-treated fat cells did not respond to glucagon but the stimulatory effects of epinephrine and fluoride were not influenced; the stimulatory effects of ACTH and secretin upon cyclase were reduced (40 and 60% respectively).⁹ The latter studies suggested that at least three of the hormone-selective sites project from the outer surface of the fat cell, and are differentially attacked by extracellular trypsin.

The present findings demonstrate the existence of a distinctive entity required for ACTH activation of adenylyl cyclase in the rat fat cell membrane. Marked changes in the response to ACTH can be achieved by alteration of glucocorticoid levels in the animal, whether by adrenalectomy or by administration of dexamethasone or cortisol, without significant influence on the sensitivity of ghosts to

epinephrine and glucagon or the common effector enzyme-adenyl cyclase. The findings that actinomycin D and cycloheximide block the effect of dexamethasone to selectively increase the response to ACTH strongly suggest that glucocorticoids regulate ACTH sensitivity by acting as inducers to promote the synthesis of the component required for ACTH action.¹⁷ The induction of ACTH sensitivity in rat fat cell membranes appears to be similar, in principle, to the induction of adaptive enzymes in mammalian cells by adrenal steroid hormones.¹⁸ The induction of specific hepatic enzymes by glucocorticoids has been found to be caused by an increase in the rate of enzyme synthesis and to be prevented by actinomycin D.¹⁹ On the assumption that glucocorticoids act at the gene locus, it would appear that glucocorticoids modify the degree of expression of the gene involved in ACTH sensitivity of the rat fat cell membrane. Thus, gene expression appears to be markedly reduced after adrenalectomy. Since dexamethasone induces supernormal sensitivity to ACTH, in intact as well as adrenalectomized rats, the gene seems to be partially expressed in normal rats, and can be more fully expressed when glucocorticoid levels are increased above normal.

The kinetics of the induction of ACTH sensitivity by glucocorticoids merit detailed study. Our present results indicate only that changes in ACTH sensitivity induced by dexamethasone, although detected in 5–8 hr, requires treatment for 40–60 hr to achieve supernormal values. The studies with hypophysectomized rats, where glucocorticoid deficiency and other hormonal deficiencies develop with time, raise the possibility that the induction phenomenon requires the participation of other hormones (hypophyseal and thyroid) acting together with glucocorticoids. The absence of supernormal ACTH sensitivity in ghosts from hypophysectomized rats after dexamethasone treatment could be explained on this basis; alternatively, this finding could result from a generalized decrease in over-all protein synthesis.

The molecular nature of the factor required for ACTH stimulation of rat ghost cyclase, induced by glucocorticoids, has not been established; it could be an ACTH discriminator or a specific coupling factor responsible for coupling the primary reaction of ACTH with discriminator to adenylyl cyclase. It will not be possible to definitively distinguish between these alternatives until the molecular units involved in the selective reception of ACTH, and its *mode* of coupling to adenylyl cyclase, will have been elucidated. Independent of these uncertainties, the present studies provide further evidence that the ACTH selectivity unit possesses distinctive molecular features, which differentiate it from other hormonal selectivity units and adenylyl cyclase itself in the plasma membrane of rat fat cells.

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¹ Butcher, R. W., G. A. Robison, J. G. Hardman, and E. W. Sutherland, *Advan. Enzyme Regul.*, **6**, 357 (1968); Robison, G. A., R. W. Butcher, and E. W. Sutherland, *Ann. Rev. Biochem.*, **37**, 149 (1968); Sutherland, E. W., G. A. Robison, and R. W. Butcher, *Circulation*, **37**, 279 (1968); Sutherland, E. W., and G. A. Robison, *Diabetes*, **18**, 798 (1969).

² Hechter, O., H.-P. Bär, M. Matsuba, and D. Soifer, *Life Sci.*, **8**, 935 (1969); Taunton, D. A., J. Roth, and I. Pastan, *J. Biol. Chem.*, **244**, 247 (1969).

³ Gilman, A. G., and T. W. Rall, *Fed. Proc.*, **25**, 617 (1966); *J. Biol. Chem.*, **243**, 5867 (1968); Pastan, I., and R. Katzen, *Biochem. Biophys. Res. Commun.*, **29**, 792 (1967).

⁴ Øye, I., and E. W. Sutherland, *Biochim. Biophys. Acta*, **127**, 347 (1966).

⁵ Savard, K., J. M. Marsh, and B. F. Rise, *Recent Progr. Hormone Res.*, **21**, 285 (1965).

⁶ Birnbaumer, L., L. S. Pohl, and M. Rodbell, *J. Biol. Chem.*, **244**, 3468 (1969); Birnbaumer, L., and M. Rodbell, *J. Biol. Chem.*, **244**, 3477 (1969).

⁷ Bär, H.-P., and O. Hechter, these PROCEEDINGS, **63**, 350 (1969).

⁸ Vaughan, M., and F. Murad, *Biochemistry*, **8**, 3092 (1969).

⁹ Rodbell, M., L. Birnbaumer, and S. L. Pohl, *J. Biol. Chem.*, **245**, 718 (1970).

¹⁰ Rodbell, M., *J. Biol. Chem.*, **239**, 375 (1964).

¹¹ Bär, H.-P., and O. Hechter, *Anal. Biochem.*, **29**, 476 (1969).

¹² Following adrenalectomy a similar selective loss of ACTH activity was observed in a "fat-free" broken cell preparation, obtained by vortex treatment of fat cells in glass, which contains all cellular constituents.

¹³ Treatment of adrenalectomized rats over 48 hr with four separate injections of dexamethasone (total dose of 0.4 mg/kg body weight) produced an eightfold enhancement of the maximal response to ACTH. Treatment with four injections of cortisol (total dose of 2 mg/kg body weight) produced only a twofold augmentation of the ACTH response.

¹⁴ Reich, E., R. M. Franklin, A. J. Shatkin, and E. L. Tatum, *Science*, **134**, 556 (1961).

¹⁵ Siegel, M. R., and H. D. Sisler, *Biochim. Biophys. Acta*, **87**, 70 (1964).

¹⁶ Actinomycin D and cycloheximide were highly toxic to adrenalectomized rats; however, the tolerance of these rats to the antibiotics was increased by simultaneous administration of dexamethasone. When dexamethasone was administered simultaneously with either actinomycin D or cycloheximide, the effect of dexamethasone to enhance the ACTH response was abolished.

¹⁷ We have considered the possibility that changes in membrane calcium might be the factor underlying the observed alterations in ACTH responsiveness, especially in the light of previous findings,^{6,7} that Ca^{2+} is required for ACTH stimulation of ghost adenylyl cyclase preparations. At the present time there is no evidence that this is the case. Addition of Ca^{2+} (0.02 mM) or Sr^{2+} (1 mM) to the assay system failed to influence the decreased ACTH response of fat cell ghosts from adrenalectomized rats. Also no difference was found in the ability of the calcium chelating agent EGTA (ethyleneglycol-bis (β -aminoethylether)- N,N' -tetraacetic acid) to abolish ACTH stimulation whether added to adenylyl cyclase ghost preparations derived from control or dexamethasone-treated rats.

¹⁸ Feigelson, P., M. Feigelson, and O. Greengard, *Recent Progr. Hormone Res.*, **18**, 491 (1962); Tomkins, G. M., L. D. Garden, R. R. Howell, and B. Peterkofsky, *J. Cell Comp. Physiol.*, **66**, 137 (1965).

¹⁹ Schimke, R. T., E. W. Sweeney, and C. M. Berlin, *J. Biol. Chem.*, **240**, 322 (1965).