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Action of Feedback Regulator on Adenylate Cyclase

[plasma membrane/adipocytes/membrane phosphorylation/adenosine 5′-(β,γ-imino)triphosphate/adenosine 5′-(α,β-methylene)triphosphate]

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ABSTRACT **A factor [the feedback regulator (FR)] formed by adipocytes after the stimulation of a cAMP raising hormone has been found to be a potent inhibitor of membrane-bound adenylate cyclase [EC 4.6.1.1; ATP pyrophosphate-lyase (cyclizing)]. In a standard assay system using rat adipocyte plasma membrane as the source of adenylate cyclase, the FR inhibited adenylate cyclase by lowering the V_{max} without affecting the apparent K_m for ATP (0.3–0.6 mM). The apparent K_a for epinephrine (5–6 μM) was also not affected by FR. The inhibitory action of FR was partially countered by Mg^{2+} ions.**

An increase in phosphorylation of plasma membrane was observed when FR was present in the incubation system. The concentration required for a 50% inhibition was four times higher when adenosine 5′-(β,γ-imino)triphosphate [AMP-P(NH)P] replaced ATP as the substrate for adenylate cyclase, implying that adenylate cyclase was inactivated by phosphorylation caused by FR. Increase in FR inhibition obtained by adding low concentrations of adenosine 5′-(α,β-methylene)triphosphate or ATP to AMP-(NH)P as the substrate supports this view. The inhibitory action was reversible. These results are consistent with the previously reported phenomena that (1) the unresponsiveness of adipocytes after hormone stimulation is due to the formation of FR, and (2) the recovery of responsiveness of the stimulated cells by washing the cells with regular buffer medium is a result of the removal of FR. The hormone-initiated biphasic curve of cAMP levels in adipocytes is believed to be due to the negative feedback action of FR on adenylate cyclase. The mechanism of action of FR on inhibition of adenylate cyclase appears to be related to the phosphorylation of certain membrane components.

We have investigated the feedback regulation of adenylate cyclase [EC 4.6.1.1; ATP pyrophosphate-lyase (cyclizing)], using plasma membrane from adipocytes as a model system. We have reported previously that a cAMP-mediated feedback regulator (FR) may play an important role in the regulation of adenylate cyclase activity (1). Some phenomena, namely the biphasic time course of cellular cAMP levels and the unresponsiveness of adipocytes to further stimulation by hormones, could be explained by the formation of FR (1). A partial purification of the FR has been reported (2).

The present study describes the possible mechanism of FR on the inhibition of hormone-stimulated adenylate cyclase. The experimental design was focused on the interaction of the FR with adenylate cyclase and its substrate (ATP), cofactor (Mg^{2+}) and stimulators (epinephrine and F^-). The reversibility of enzymatic activity after FR treatment and the relationship between the phosphorylation of components of the plasma membrane and adenylate cyclase are described.

Abbreviations: FR, feedback regulator; AMP-P(NH)P, adenosine 5′-(β,γ-imino)triphosphate; AMP-(CH_2)PP, adenosine 5′-(α,β-methylene)triphosphate.

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MATERIALS AND METHODS

Materials and methods used were essentially the same as those previously described (1).

Preparation of the Membrane-Bound Hormone-Sensitive Adenylate Cyclase. The membrane-bound hormone-sensitive adenylate cyclase was prepared from isolated adipocytes of rat by the method of Laudat *et al.* (3). Normally fed albino rats of the Sprague-Dawley strain, weighing approximately 150–200 g, were used throughout the experiments. The yield of membrane protein of adipocytes from 50 rats was 9.9 ± 1.42 mg (mean ± SEM for 9 experiments), measured by the method of Lowry *et al.* (4). The epinephrine-sensitive adenylate cyclase activity was 8.1 ± 1.5-fold above the basal activity. In some cases the plasma membrane was prepared from recovered adipocytes after the preparation of FR (see below). The hormone sensitivities of membrane adenylate cyclases from both fresh and recovered cells were identical.

Preparation of FR. The FR was prepared and purified from adipocytes after the stimulation by a lipolytic hormone and/or other agents (1) as has been previously described. The active components of FR in the salt-free dried albumin mixture were stable indefinitely at −20°. FR could be further purified by ethanol extraction and by chromatography on Sephadex LH-20 columns, and was separated from added free fatty acids, glycerides, phospholipids, and prostaglandin E_1 on a Sephadex LH-20 column eluted with a mobile phase containing acetone–ethanol–heptane–water (40:50:1:9 v/v).

Assay of Adenylate Cyclase. This procedure was essentially according to the method of Rall and Sutherland (5) with minor modifications. The assay mixture consisted of 2 mM ATP, 4 mM Mg^{2+}, 6 mM theophylline, 230 μM epinephrine, 40 mM glycylglycine buffer, pH 7.4, 20–40 μg of rat adipocyte membrane protein, and partially purified FR. The final volume was 500 μl or as otherwise specified. The reaction was carried out at 30° for 20 min. cAMP formed in the assay system was chromatographically isolated and was estimated by the protein binding assay of Gilman (6).

Phosphorylation of Plasma Membrane. About 10^6 cpm of [γ-^{32}P]ATP was added to the adenylate cyclase assay system which contained 2 mM ATP, 4.0 mM Mg^{2+} in glycylglycine buffer, pH 7.4, with no caffeine present. The final volume was 80 μl. The tubes were incubated at 30° for 5 min and the reaction was stopped by applying an aliquot from each tube to filter paper (2 cm^2 Whatman no. 3), which was placed im-

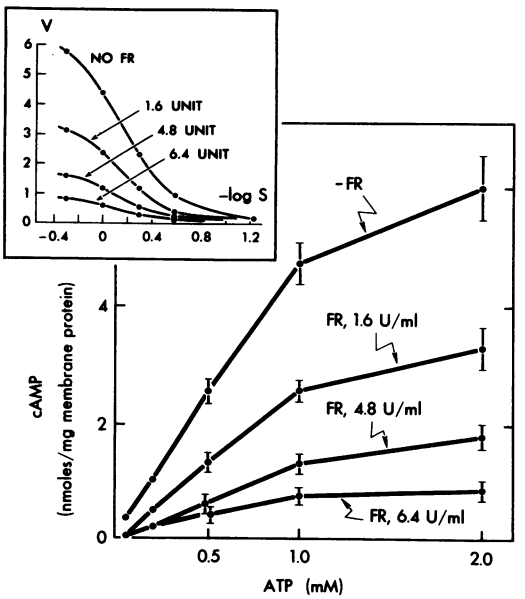

FIG. 1. Inhibition of adenylate cyclase by FR at various concentrations of ATP. All samples were incubated at 30° for 15 min. Results are the mean of four incubations and the vertical lines represent the standard error of the mean. The insert is a plot of V versus $-\log S$ (11) with the same units of V and S.

mediately into a beaker containing 200 ml of cold 10% trichloroacetic acid (7). Following two rinses with the same acid and two rinses with methanol, the radioactivity of the filter paper was estimated in a Packard Tri-Carb liquid scintillation spectrometer (model 3390) in 10 ml of scintillation medium with toluene-Triton X-100 as solvent (8). In recent experiments the reaction was stopped by addition of 100 μl of 10% cold trichloroacetic acid. The reaction mixture was transferred to a Millipore filter while the vacuum pump was running. The tube was washed with 1 ml of 20 mM potassium phosphate buffer, pH 7.4, with 1 mM EDTA, and the filter was washed with 25 ml of the same buffer. The Millipore filter was transferred to a scintillation counting vial and 10 ml of scintillation medium was added. The radioactivity was then estimated. The results obtained with this method were identical with those of the filter paper method.

TABLE 1. *The effect of FR on epinephrine-stimulated adenylate cyclase activity*

FR, U/ml	Cyclase activity, nmol/mg membrane protein	% Inhibition
0	6.55 ± 0.35	0
0.32	5.70 ± 0.25	13
0.64	4.97 ± 0.20	24
2.0	3.27 ± 0.21	50
3.28	1.84 ± 0.08	72
5.66	1.05 ± 0.16	84
6.70	0.85 ± 0.05	87

The adenylate cyclase assay of adipocyte plasma membrane was adopted to measure FR. The incubation system consisted of 2 mM ATP, 4 mM Mg^{2+}, 6 mM theophylline, 230 μM epinephrine, 50 mM glycylglycine, pH 7.4, and 28 μg of membrane protein, with or without FR. The final volume was 500 μl. The incubation was carried out at 30° for 15 min. The reaction was stopped by the addition of perchloric acid to a final concentration of 0.3 M. Results are expressed as both nmol of cAMP/mg of membrane protein per 15 min and as % inhibition. The unit of FR was defined as that amount of FR which caused a 50% decrease in the hormone-stimulated adenylate cyclase of this assay system. Each point is a mean of four incubations ± SEM. The % inhibition was calculated as follows: [(cAMP, without FR − cAMP, with FR) × (cAMP, without FR)$^{-1}$] × 100.

Other Materials. *l*-Epinephrine hydrochloride (adrenalin) was supplied by Parke-Davis, Detroit, Mich. Collagenase was purchased from Worthington Biochemical Corp., Freehold, N.J. Bovine serum albumin fraction V was purchased from Nutritional Biochemical Corp., Cleveland, Ohio, and defatted in our own laboratories (9). Tritiated cAMP ([*G*-^{3}H]cAMP, 23 Ci/mmol) was purchased from New England Nuclear and purified by anion exchange chromatographic procedures (1). [γ-^{32}P]ATP was prepared by the enzymatic method of Glynn and Chappell (10). Carrier-free $^{32}PO_4$ was purchased from International Chemical and Nuclear Corp., Irvine, Calif. Adenosine 5′-(β,γ-imino)triphosphate [AMP-P(NH)P] was purchased from ICN Pharmaceuticals, Inc., Irvine, Calif. Adenosine 5′-(α,β-methylene)triphosphate [AMP-(CH$_2$)PP] was obtained from P-L Biochemicals, Inc., Milwaukee, Wisc. as the lithium salt, approximately 98% pure.

RESULTS

Inhibition of Epinephrine-Stimulated Adenylate Cyclase Activity by FR in the Presence of Increasing Concentrations of FR. Adenylate cyclase of adipocyte plasma membrane stimulated by maximum concentrations of epinephrine was inhibited by FR. The degree of inhibition was progressively increased as the relative concentration of FR increased. The unit of FR was arbitrarily assigned as the amount of active FR that inhibited 50% of the epinephrine-stimulated adenylate cyclase activity at a defined condition. Results are shown in Table 1.

Inhibition of Membrane-Bound Adenylate Cyclase Activity by FR in the Presence of Increasing Concentrations of Epinephrine. In a standard assay system, adenylate cyclase of adipocyte plasma membrane was stimulated as the concentration of epinephrine increased up to 100 μM (results not shown). Higher concentrations of epinephrine caused no further increase in adenylate cyclase activity. The maximum stimulation of adenylate cyclase activity was about 12-fold above the basal level at 0.1 mM epinephrine. The apparent K_a for epinephrine was approximately 5.9 μM. Epinephrine-stimulated cAMP formation was inhibited with increasing concentrations of FR. FR, 1.6 U/ml, inhibited about 44% at each concentration of epinephrine tested; 4.8 U/ml inhibited 75%. The data was analyzed in two ways: Lineweaver-Burk plot and Hofstee plot (11). The results indicate that FR-inhibited epinephrine-stimulated adenylate cyclase shows noncompetitive kinetics. Thus it is evident that FR decreased the V_{max} of cAMP formation without significantly altering the apparent K_a for epinephrine. Furthermore, the basal adenylate cyclase activity was also inhibited 17% by 1.6 U/ml.

Inhibition of Adenylate Cyclase by FR in the Presence of Varied Concentrations of ATP. As seen in Fig. 1, when the concentration of the substrate ATP was a variable in the assay system and the molar Mg^{2+} to ATP ratio was fixed at 2, inhibition was dependent on the dose of FR. The inhibition of cAMP formation by 1.6, 4.8, and 6.4 U/ml of FR at an ATP substrate level of 2.0 mM, was 44%, 70%, and 85%, respec-

tively. The percentage inhibition with 1.6 U/ml of FR was 45% and not significantly different over a wide range of ATP concentrations from 0.2 to 2 mM. The inhibition by FR was also noncompetitive with respect to ATP as judged by the kinetic analysis in that V_{max} values were affected but the apparent K_m for ATP (0.55 mM) was not modified by FR (Fig. 1, insert).

Effect of Mg^{2+} Concentration on the Inhibition of Adenylate Cyclase by FR. The effect of FR on plasma membrane adenylate cyclase was tested with various Mg^{2+} concentrations. ATP concentration was 2 mM for all tubes. The results indicate (Fig. 2) that adenylate cyclase activity was increased about 2.5-fold if Mg^{2+} was increased from 2 to 20 mM. Addition of FR to the cyclase system caused a decrease of cAMP formation at each Mg^{2+} concentration tested. However, the inhibitory activity was more pronounced at lower (2–4 mM) Mg^{2+} concentrations. With the addition of FR, 4 U/ml, a 73% inhibition of cAMP production was observed. If Mg^{2+} was increased to 20 mM, the same concentration of FR inhibited only 44% of cAMP formation. If FR concentration was increased to 6 U/ml the inhibitory action on cAMP formation was 97% and 73% for the Mg^{2+} concentrations of 4 mM and 20 mM.

Plots of $(1/V)$ versus $(1/[Mg^{2+}])$ with or without FR in the assays intercept at the ordinate (11). The apparent V_{max} is 25 nmol/mg per 15 min and the apparent K_m is 5 and 20 mM Mg^{2+} without and with FR, respectively. These results suggest that FR is a competitive inhibitor of Mg^{2+} under the conditions used (Fig. 2, insert). However, other data show that the activity of adenylate cyclase was not further increased when Mg^{2+} concentrations were higher than 20 mM, and the competition of FR with respect to Mg^{2+} was no longer complete when Mg^{2+} concentration was increased to 80 mM. These results are complicated by the fact that Mg^{2+} at 80 mM inhibited adenylate cyclase activity.

Comparison of the Inhibitory Action of FR on Hormone-Stimulated and Fluoride-Stimulated Adenylate Cyclase. The hormone-stimulated and fluoride-stimulated adenylate cyclase activities from several different preparations (adipose tissue homogenates, washed particles from adipose tissue homogenates, and purified adipocyte plasma membrane) were inhibited by FR. The degrees of inhibition by FR of hormone- and fluoride-stimulated adenylate cyclase activity were studied and found to be different. One unit of FR, which by definition inhibits the epinephrine-stimulated adenylate cyclase 50% in this system (see also Table 1), inhibited the fluoride-stimulated adenylate cyclase only 15% (results not shown).

Comparison of the Inhibitory Action of FR on Adenylate Cyclase Assayed in the Presence of ATP, AMP-P(NH)P and AMP-(CH_2)PP. Adenylate cyclase of adipocyte plasma membrane can use AMP-P(NH)P as substrate and can be enhanced by GTP or ATP as in the case of liver plasma membrane described by Rodbell *et al.* (12, 13).

Inhibitory action by FR on adenylate cyclase with different substrates is compared in Fig. 3. In the presence of ATP as substrate, the FR required for a 50% inhibition of adenylate cyclase was about 2 U/ml; when AMP-P(NH)P (pH 7.4) was used as substrate, 8 U/ml of FR was required. Addition of 10 μM ATP to AMP-P(NH)P increased the inhibitory action of FR and only 5.8 U/ml was required for 50% inhibition (Fig. 3).

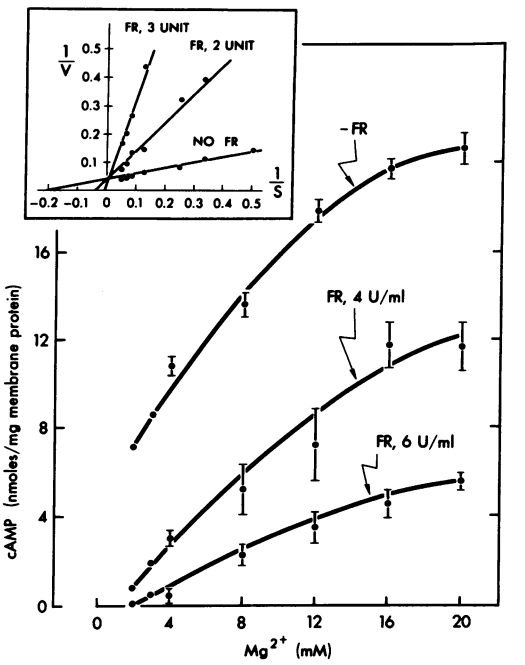

FIG. 2. The effect of Mg^{2+} concentration on the inhibition of adenylate cyclase activity by FR. All samples were incubated at 30° for 15 min. Results are the mean of four incubations and the vertical lines represent the standard error of the mean. The insert is a Lineweaver-Burk plot (11).

When AMP-(CH_2)PP, an ATP analogue, was used in combination with AMP-P(NH)P in an adenylate cyclase assay system, Amp-(CH_2)PP mimicked the action of ATP as sub-

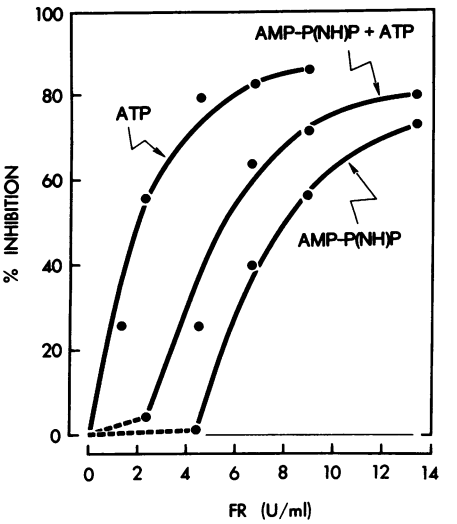

FIG. 3. Inhibition of plasma membrane adenylate cyclase activity by FR with AMP-P(NH)P and/or ATP as substrate. Plasma membrane adenylate cyclase was measured according to the method described in *Materials and Methods* section. When ATP was used as substrate, the concentration of ATP was 2 mM and Mg^{2+} concentration was 4 mM. When AMP-P(NH)P was used as substrate, the concentration of AMP-P(NH)P was 0.7 mM and Mg^{2+} concentration was 1.4 mM. The assay pH was 7.4. When both ATP and AMP-P(NH)P were used, the concentration of AMP-P(NH)P was 0.7 mM and that of ATP was 10 μM; Mg^{2+} concentration was 1.4 mM. Samples containing no FR were assigned at 0% inhibition. A unit of FR was determined by a standard adenylate cyclase assay.

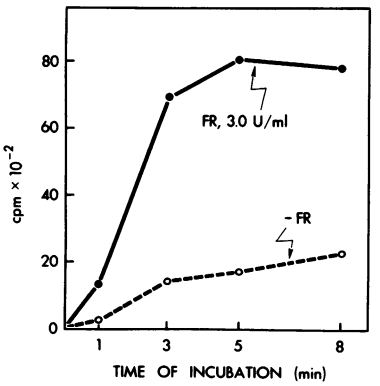

FIG. 4. The effect of FR on the incorporation of γ-phosphate of ATP into adipocyte membrane. Plasma membrane (12 μg) was incubated in a system similar to that used for adenylate cyclase with a final volume of 80 μl, but in the absence of theophylline and in the presence of 1×10^6 cpm of [γ-^{32}P]ATP. The reaction was terminated and phosphorylated protein was measured by a method described by Hayakawa for protein kinase assay (7).

strate (results not shown). FR at 2.4 U/ml inhibited adenylate cyclase by 27% in the absence of AMP-(CH_2)PP and by 47% in the presence of AMP-(CH_2)PP. The AMP-(CH_2)PP effect was 27%.

Effect of FR on the Incorporation of γ-Phosphate of ATP by Adipocyte Plasma Membrane. In addition to inhibiting adenylate cyclase activity, FR was shown to stimulate the catalytic subunit of cAMP-dependent protein kinase (14), suggesting that FR may inhibit adenylate cyclase by a mechanism involving phosphorylation. As shown in Fig. 4, there was a time-dependent incorporation of the γ-^{32}P from labeled ATP in the absence of FR. Addition of 3.0 U/ml of FR caused a 4-fold stimulation of the incorporation.

Reversible Inhibition by FR of Hormone-Stimulated Adenylate Cyclase of Adipocyte Plasma Membrane. It has been shown in a previous report (1) that the declining portion of the biphasic curve of the cAMP levels in adipocytes, after hormone stimulation, was the result of the formation of a regulator. This endogenous regulator caused a decrease in hormone action or unresponsiveness of the adipocyte to a second hormone stimulation. The hormone responsiveness could be restored if the unresponsive adipocytes were washed three times with warm Krebs-Ringer bicarbonate buffer. We can now show that the plasma membrane of fresh or recovered adipocytes was responsive to epinephrine, adrenocorticotropic hormone, and glucagon. Reversal of the inhibitory action by FR on plasma membrane adenylate cyclase was also demonstrable. Table 2 shows that incubation of membranes with 2 mM ATP, 4 mM Mg^{2+}, and 1.1 U/ml of FR resulted in 38% inhibition of cAMP formation. This inhibited membrane can recover its hormone responsiveness by being washed in 10 volumes of a medium containing 10 mM Tris·HCl and 1 mM EDTA, pH 7.4, at 4°. The hormone response was completely recovered.

TABLE 2. *Reversible inhibition by FR of membrane adenylate cyclase*

Experiment	FR during pre-incubation, 1.1 U/ml	FR during assay, 1.1 U/ml	Epinephrine, 460 μM	Adenylate cyclase, nmol/mg of protein per 15 min
A	No pre-incubation	—	—	1.10 ± 0.03
	No pre-incubation	—	+	8.65 ± 0.41
	No pre-incubation	+	—	1.00 ± 0.02
	No pre-incubation	+	+	5.36 ± 0.04
B	—	—	—	1.33 ± 0.06
	—	—	+	8.42 ± 0.49
	+	—	—	1.31 ± 0.04
	+	—	+	9.84 ± 0.11

In experiment A, plasma membrane, 20 μg, was used for the assay of adenylate cyclase without pre-incubation. In experiment B, 200 μg of plasma membrane were incubated in a glycylglycine buffer, 50 mM, pH 7.4, with or without FR (1.1 U/ml) and with ATP, 2.0 mM, Mg^{2+}, 4 mM, without epinephrine for 4 min at 30°. Incubation volume was 1 ml. Following incubation, the membrane was washed with 10 ml of cold buffer medium containing 10 mM Tris and 1 mM EDTA, pH 7.4, and centrifuged at 10,000 × *g* for 10 min. Then the membrane was resuspended in the same buffer to the original volume. Aliquots of 100 μl were used for adenylate cyclase assay and protein measurement [Lowry *et al.* (4)].

DISCUSSION

In our previous reports (1, 2) we indicated that the inhibitory action of FR on the hormone-stimulated rise of cAMP levels in adipocytes appeared to be nonspecific for the hormone used. The inhibition by FR of such a rise of cAMP levels is now shown to be a result of inhibition of cAMP synthesis. This inhibition of adenylate cyclase could not be overcome by increasing hormone concentration. Using purified plasma membrane as an assay system for adenylate cyclase (as in the present system), one must remember that there are several enzymes which form a complex with the matrix of the membrane (15). The membrane bound enzymes such as ATPase, phosphodiesterase, and protein kinase as well as hormone-sensitive adenylate cyclase deserve primary consideration. Therefore, the action of FR in the inhibition of cAMP formation in the assay system is by no means established as a direct inhibition of adenylate cyclase. The possible sites of action may be narrowed, however, as the results of this study suggest.

The observation that the inhibition of cAMP formation by FR is not affected by varying the ATP concentration suggests that the inhibitory mechanism cannot be explained by a reduction of the concentration of the substrate ATP, therefore, an increase of ATPase activity by FR is unlikely. Furthermore, ATPase activity was found to be slightly inhibited by FR (unpublished observation).

It is unlikely that FR caused less apparent cAMP accumulation as a result of increased activity of membrane bound phosphodiesterase. The presence of adequate concentrations of phosphodiesterase inhibitor and evidence of inhibitory action of FR on membrane phosphodiesterase (16) argue against this view.

Mg^{2+}–ATP ratios are known to influence the velocity of the adenylate cyclase reaction (17, 18) and an excess of free Mg^{2+} over ATP is required for optimal activity of this enzyme. In the present study the FR effect on the percent of inhibition was gradually reduced as the concentration of Mg^{2+} was increased up to 20 mM. Whether interactions of FR with enzyme affect the binding of free Mg^{2+} to adenylate cyclase is not yet clear. A direct binding of Mg^{2+} to FR is unlikely, since there are other Mg^{2+}-dependent reactions which are stimulated by FR (14). The intracellular free Mg^{2+} concentra-

tion is much lower [0.6–1.3 μmol/g of wet weight (19)] than the free Mg^{2+} concentration that was used in the assay. Therefore, FR could be even more effective intracellularly.

A possible mechanism of action of FR is suggested by the finding that the inhibitory action of FR was greatly reduced when AMP-P(NH)P was used as the substrate. Since AMP-P(NH)P is neither a substrate for ATPase (12, 17) nor a phosphate donor (21), the results suggest that either FR increased ATPase activity or ATP is required for FR action. The latter possibility appeared to be more likely and was further supported by the results obtained with AMP-(CH_2)PP and AMP-P(NH)P. AMP-(CH_2)PP is another analogue of ATP with a transferable γ-phosphate but it is not a substrate for adenylate cyclase. It appears that the optimal effect of FR requires a transferable phosphate group, the γ-group of ATP. This theory was further strengthened by phosphate incorporation experiments in which FR increased [^{32}P]phosphate incorporation onto plasma membrane with [γ-^{32}P]ATP as the substrate. However, the inhibition of adenylate cyclase observed with AMP-P(NH)P at high FR concentrations remains to be explained. Since AMP-P(NH)P is not a phosphate donor in the kinase reactions (21), it is possible that FR could have two effects: (*1*) to promote the phosphorylation of the cyclase, and (*2*) to inhibit dephosphorylation of the cyclase.

Since plasma membranes of several sources contain both cAMP-dependent and cAMP-independent protein kinase activities, as reported by Lemay *et al.* (22), the increase in phosphorylation of adipocyte membrane could be explained by the fact that under certain conditions FR stimulated protein kinase activity (R. J. Ho, T. Soderling, and E. W. Sutherland, unpublished observations). However, the phosphorylation of plasma membrane may or may not be related to the inhibitory action of FR on adenylate cyclase. The possibility that inhibitory action on adenylate cyclase is a result of an increase in phosphorylation of this enzyme is partially negated by the evidence that the onset of inhibitory action is instantaneous while the phosphorylation of the membrane progressively increases, reaching its peak at 5 minutes. The phosphorylation of adenylate cyclase may be rapid, however, while protein of the membrane other than adenylate cyclase may be more slowly phosphorylated. A phosphorylated form of adenylate cyclase may be an inactive form as suggested by Najjar *et al.* (23). Our evidence, obtained from AMP-P(NH)P and the enhancement of inhibitory action by ATP and AMP-(CH_2)PP seems to support this view. The smaller degree of inhibition of fluoride-stimulated adenylate cyclase by FR may also be consistent with the phosphorylation theory in that F^- might increase the dephosphorylation of the adenylate cyclase (23). However, the mechanism of action of F^- ion on adenylate cyclase has not yet been elucidated (24–26).

The reversibility of FR action on adenylate cyclase was demonstrated with several conditions: (*1*) the recovered cell had normal hormone responsiveness (1); (*2*) plasma membrane from stimulated adipocytes had normal hormone responsiveness (R. J. Ho and E. W. Sutherland, unpublished data); and (*3*) the inhibited membrane adenylate cyclase was deinhibited following adequate washes with a defined medium.

The reversible effect of FR meets the physiological requirements for a regulator, and is consistent with our previous finding of nonresponsiveness and restoration of responsiveness of adipocytes after hormone stimulation and washing (1). A dephosphorylation step may take place during the restoration of the hormone sensitivity. The mechanism of action of FR and the reversibility of adenylate cyclase inhibition cannot be clearly understood until the hormone-sensitive adenylate cyclase system is purified.

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