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Stimulation of a Low K_m GTPase by Inhibitors of Adipocyte Adenylate Cyclase

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SUMMARY

The activity of enzymes exhibiting GTPase activity was determined in membrane preparations of hamster adipocytes. Two GTPases with apparent K_m values of about 0.2 μM GTP (low K_m GTPase) and 180 μM GTP (high K_m GTPase) were found. The effects of various agents that stimulate or inhibit adipocyte adenylate cyclase were investigated with these two forms of GTPase. None of the stimulatory or inhibitory agonists studied affected the activity of the high K_m GTPase(s). However, factors inhibiting adenylate cyclase, such as prostaglandin E_1, nicotinic acid, 3-carboxy-5-methylpyrazole, and N^6-phenylisopropyladenosine, stimulated the low K_m GTPase by 50–100% without an apparent lag phase. The activity of the stimulated GTPase was half-maximal at about 0.2 μM GTP. NaCl (up to 100 mM) had no effect on the basal activity of this enzyme but amplified the stimulation induced by adenylate cyclase inhibitory agents. There was a good correlation between inhibition of adenylate cyclase and stimulation of the low K_m GTPase, both with regard to the concentration required for half-maximal effects on these two enzymes and with regard to the potency order of various prostaglandins studied. In contrast to factors inhibiting adenylate cyclase, the stimulatory hormones, isoproterenol and ACTH, had only small effects on the low K_m GTPase activity; potassium fluoride was completely ineffective. The data suggest that an increased GTP hydrolysis by an activated GTPase is an essential mechanism of hormone-induced adenylate cyclase inhibition.

INTRODUCTION

Hormone-sensitive adenylate cyclase [ATP pyrophosphate-lyase (cyclizing); EC 4.6.1.1] is a multi-component system consisting at least of the hormone receptor, the catalytic subunit, and a regulatory guanine nucleotide-binding protein (2). Although the exact mechanisms of hormone-induced stimulation of adenylate cyclase are not fully understood, it is thought that occupation of the receptor by a stimulatory hormone promotes the exchange of GDP by GTP at the regulatory site; interaction of the regulatory protein in its GTP-bound form with the catalytic component activates the cyclase (2–4). According to the regulatory GTPase cycle proposed by Cassel and Selinger (4) and Cassel *et al.* (5), activation is turned off by hydrolysis of bound GTP to GDP and P_i by a GTPase activity apparently associated with the regulatory protein. Several findings are compatible with this model: for instance, nonhydrolyzable GTP analogues such as 5′-guanylyl imidodiphosphate can cause persistent adenylate cyclase activation in various tissues studied (6). Furthermore, cholera toxin, which activates adenylate cyclase in the presence of GTP almost to the same extent as do stable GTP analogues, has been shown to inhibit a low K_m, hormone-sensitive GTPase in turkey erythrocytes (7), apparently by an ADP-ribosylation of a component of the regulatory protein (8, 9).

In contrast to hormone-induced stimulation, the mechanisms of negative control of adenylate cyclase activity by hormonal factors, such as *alpha*-adrenergic and muscarinic cholinergic agonists, opiates, adenosine, or prostaglandins, are still obscure (10). It has been shown that GTP, which is required for hormone-induced stimulation of adenylate cyclase (2), is also essential for inhibition of the enzyme by hormonal factors (10–16). In contrast to the stimulatory hormonal regulation, stable GTP analogues reversed or prevented hormone-induced inhibition of adenylate cyclase in various tissues (10, 13, 15, 17–19), including hamster adipocytes (20).

These data prompted us to study the possible role of a GTPase in the negative hormonal control of adenylate cyclase activity in hamster adipocytes. We report here that factors inhibiting adenylate cyclase stimulate a low K_m GTPase in membrane preparations of hamster adipocytes and that there exists a good correlation between stimulation of the GTPase and inhibition of the adenyl-

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ate cyclase induced by these agents. These findings suggest that stimulation of the GTPase is an essential mechanism of hormone-induced adenylate cyclase inhibition.

MATERIALS AND METHODS

Materials. The PGs[1] E_1, E_2, D_2, and $F_{2\alpha}$ were gifts of Dr. J. Pike, The Upjohn Company (Kalamazoo, Mich.); 3-carboxy-5-methyl-pyrazole, of Dr. W. Losert, Schering (Berlin, Federal Republic of Germany); N^6-phenylisopropyladenosine, of Boehringer Mannheim (Mannheim, Federal Republic of Germany); and the phosphodiesterase inhibitor Ro 20-1724, of Hoffmann-La Roche (Nutley, N. J.). App(NH)p, ATP (essentially free of GTP), ITP, CTP, UTP, ADP, GDP, and adenosine deaminase were purchased from Boehringer Mannheim. All other reagents were obtained as previously described (20). [α-^{32}P]ATP and [γ-^{32}P]GTP were prepared according to the method of Walseth and Johnson (21) and had specific activities of more than 100 Ci/mmole. Preparation of hamster adipocyte ghosts was carried out as described before (20); the ghosts were stored in small aliquots at −85°. Protein was determined by the technique of Lowry *et al.* (22). Nucleotides were separated by chromatography on polyethyleneimine cellulose plates (E. Merck, Darmstadt, Federal Republic of Germany). Separation was performed using successively 0.5 M $(NH_4)_2SO_4$ (running distance 4 cm) and 0.7 M $(NH_4)_2SO_4$ (running distance 10 cm) (23). The UV-absorbing areas were cut out and placed in scintillation vials containing water, and radioactivity was determined using Čerenkov radiation. The R_F values were 0.18, 0.27, 0.33, and 0.42 for GTP, ATP, GDP, and ADP, respectively.

GTPase assay. GTPase activity was determined according to the method of Cassel and Selinger (23). The assay medium, if not otherwise stated, consisted of 0.3 μM [γ-^{32}P]GTP (0.05–0.2 μCi/tube), 2 mM $MgCl_2$, 0.1 mM ATP, 3 mM App(NH)p, 5 mM creatine phosphate used as its tris(hydroxymethyl)aminomethane salt, creatine kinase (1.2 mg/ml), 1 mM 3-isobutyl-1-methylxanthine, 1 mM EDTA, 1 mM dithiothreitol, 100 mM NaCl, 0.2% (w/v) bovine serum albumin, and 50 mM triethanolamine-HCl (pH 7.4) in a final volume of 100 μl. Reactions were started by the addition of adipocyte ghosts (3–10μg of protein) to the prewarmed reaction mixture and conducted for 5 min or as indicated at 25°. The reactions were terminated by the addition of 700 μl of ice-cold sodium phosphate buffer (20 mM, pH 7.0) containing 5% (w/v) activated charcoal. Reaction tubes were centrifuged for 10 min at 10,000 × *g*, and 500 μl of the supernatant were transferred into scintillation vials containing 10 ml of water. Radioactivity was determined by measuring Čerenkov radiation. Release of $^{32}P_i$ in the absence of membrane preparations was 0.5–1% of added [γ-^{32}P]GTP.

Adenylate cyclase assay. Adenylate cyclase activity was determined under conditions identical with those described above for measurement of GTPase activity, with the exception that App(NH)P was omitted and that 0.3 μM unlabeled GTP and 0.1 mM [α-^{32}P]ATP (0.4–0.6 μCi/tube) were present. Reactions were started by the addition of adipocyte ghosts (10–25 μg of protein) and conducted for 5 min at 25°. Termination of the reactions and separation of cyclic AMP formed were performed by a combined $ZnCO_3$ precipitation and chromatography on neutral alumina as described (24). Both assays were performed in triplicate with intra-assay variations of less than 5% of the means. Results similar to those shown were obtained in experiments repeated several times.

RESULTS

The hydrolysis of [γ-^{32}P]GTP by the adipocyte membrane preparation used was due to at least two GTPase activities. The liberation of $^{32}P_i$ from [γ-^{32}P]GTP was effectively reduced by low concentrations of unlabeled GTP, as shown by the isotope dilution curve (Fig. 1). At 5 μM GTP, hydrolysis of [γ-^{32}P]GTP was reduced by about 60% and a plateau was obtained, indicating the existence of a high-affinity GTPase with a K_m value of about 0.2 μM. In the presence of excess unlabeled GTP, the hydrolysis of [γ-^{32}P]GTP was due to the activity of GTPase(s) with low affinity for GTP (apparent K_m about 180 μM and V_{max} about 20 nmoles/mg of protein per minute, derived from Lineweaver-Burk analysis). The activity of the high K_m GTPase(s) increased linearly with increasing GTP concentrations (not shown). The activity of the specific low K_m GTPase was calculated as described previously (23) by the difference between [γ-^{32}P]GTP hydrolysis measured at a low (e.g., 0.3 μM) GTP concentration and the [γ-^{32}P]GTP hydrolysis measured in the presence of 30 μM unlabeled GTP. When nucleotides were separated by thin-layer chromatography after 10 min of incubation without or with added membranes (5 μg of protein), about 93% of the total ^{32}P in nucleotides was found in GTP, whereas only about 4, 2, and 1% were

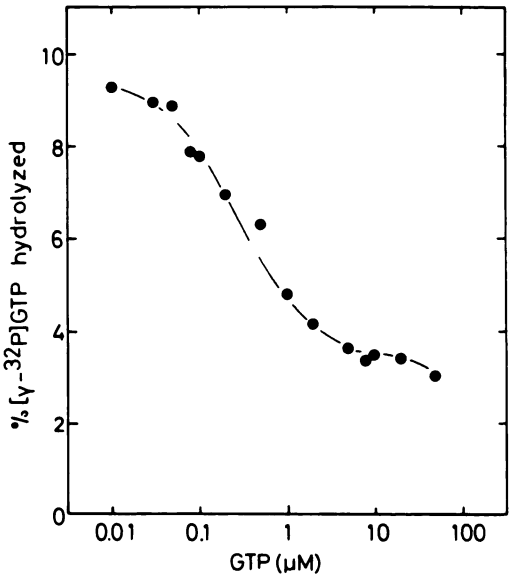

FIG. 1. *Hydrolysis of [γ-^{32}P]GTP by hamster adipocyte membranes at various concentrations of GTP*

In the presence of a constant amount of [γ-^{32}P]GTP (120,000 cpm), various amounts of GTP were added to give the final concentrations indicated on the abscissa. The percentage of [γ-^{32}P]GTP hydrolyzed is given on the ordinate.

[1] The abbreviations used are: PG, prostaglandin; App(NH)p, 5′-adenylyl imidodiphosphate.

in ATP, GDP, and ADP, respectively. These values are similar to those reported for turkey erythrocyte membranes (23).

Shown in Fig. 2 is the influence of PGE_1 and nicotinic acid on the release of $^{32}P_i$ from [γ-^{32}P]GTP by the hamster adipocyte ghosts, measured at 0.3 and 30 μM GTP. At 30 μM GTP, at which concentration only the high K_m GTPase activity is detected, neither PGE_1 (1 μM) nor nicotinic acid (30 μM; not shown) had any effect. However, at the low GTP concentration (0.3 μM), a stimulation of [γ-^{32}P]GTP hydrolysis by PGE_1 and nicotinic acid was observed. That a specific GTPase but not an unspecific phosphohydrolase or an ATPase was stimulated is indicated by the following findings. Additions of some other nucleoside triphosphates (30 μM) instead of GTP had little (UTP) or no (CTP) effect; only ITP, as shown in other GTP-utilizing systems, affected GTP hydrolysis almost as effectively (about 75%) as GTP (30 μM). Furthermore, an increase in the ATP concentration from 0.1 to 1 mM or leaving ATP out of the assay system did not change the rate of GTP hydrolysis by the low K_m enzyme. PGE_1 and nicotinic acid increased the [γ-^{32}P]GTP hydrolysis by about 20%. After subtraction of the portion of [γ-^{32}P]GTP hydrolysis due to the high K_m GTPase(s), the increase in the low K_m GTPase activity caused by PGE_1 and nicotinic acid amounted to about 80–100%. The increases in GTP hydrolysis due to PGE_1 and nicotinic acid occurred without an apparent lag phase.

We have previously shown (14, 20) that hormonal inhibition of adenylate cyclase in adipocyte membrane preparations is largely amplified by sodium ions, with a maximal effect at about 100 mM. Therefore, the influence of NaCl on the low K_m GTPase activity was studied. NaCl did not affect basal GTPase activity up to about 100 mM; at higher concentrations, NaCl reduced the enzyme activity (Fig. 3). However, stimulation of GTPase

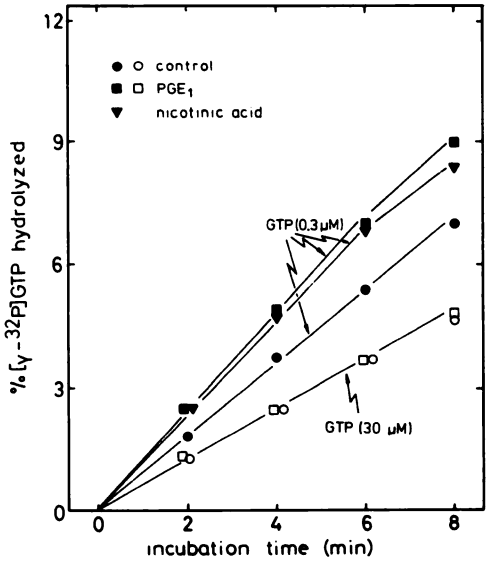

FIG. 2. *Effects of PGE_1 and nicotinic acid on the time course of [γ-^{32}P]GTP hydrolysis in adipocyte membranes measured at 0.3 and 30 μM GTP*

[γ-^{32}P]GTP hydrolysis was determined as described under Materials and Methods. *Open symbols* indicate hydrolysis of [γ-^{32}P]GTP at 30 μM and *closed symbols* at 0.3 μM GTP. The difference between hydrolysis of [γ-^{32}P]GTP measured at the low (0.3 μM) GTP concentration and that measured at the high (30 μM) GTP concentration is due to the low K_m GTPase activity as described under Results. ○●, Control; □■, PGE_1 (1 μM); ▼, nicotinic acid (30 μM).

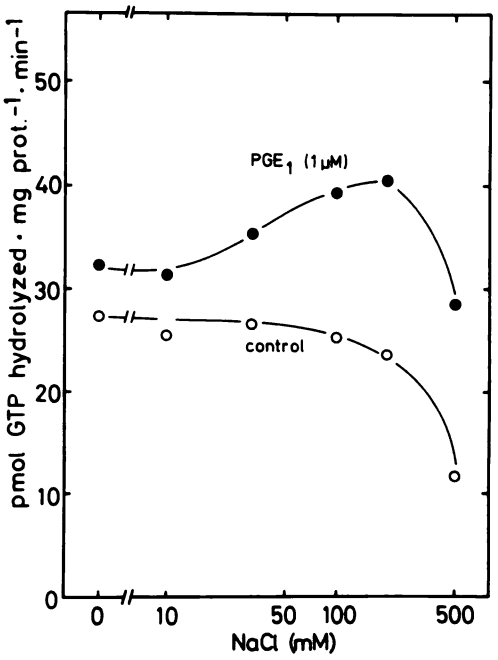

FIG. 3. *Influence of NaCl on basal and PGE_1-stimulated low K_m GTPase activity*

Low K_m GTPase activity was determined at increasing concentrations of NaCl in the absence (○) and presence (●) of PGE_1 (1 μM) and estimated as described in the legend to Fig. 2. The GTP concentration was 0.3 μM.

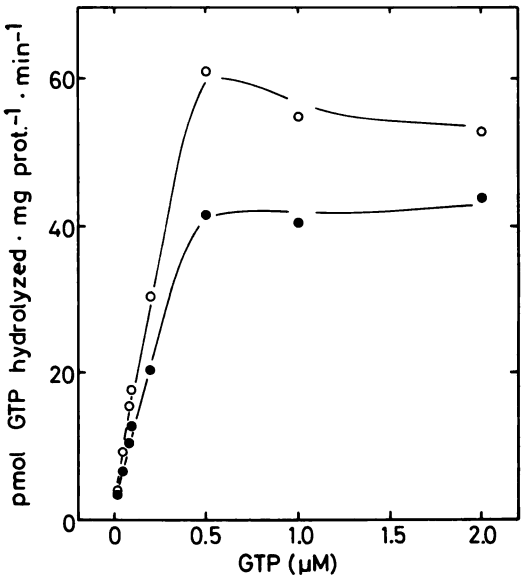

FIG. 4. *Influence of GTP on basal and PGE_1-stimulated low K_m GTPase activities*

Low K_m GTPase activity was determined at increasing concentrations of GTP as indicated on the abscissa in the absence (●) and presence (○) of PGE_1 (1 μM) and estimated as described in the legend to Fig. 2.

activity by PGE_1 (1 μM) was increased by NaCl. A maximal increase in the PGE_1-induced stimulation occurred at 100–200 mM NaCl. At 500 mM NaCl, stimulation by PGE_1 decreased, although the increment in activity over basal activity remained almost constant. Findings similar to those obtained with PGE_1 were obtained with nicotinic acid (not shown). Therefore, in all other experiments shown, NaCl (100 mM) was included in the GTPase assay medium.

The influence of GTP at various concentrations on basal and PGE_1-stimulated low K_m GTPase activities is shown in Fig. 4. At about 0.5 μM GTP, basal GTPase activity reached V_{max} with about 40 pmoles of GTP hydrolyzed per milligram of protein per minute; half-maximal activity was obtained at about 0.2 μM GTP. PGE_1 (1 μM) stimulated the low K_m GTPase at all GTP concentrations studied. In the presence of PGE_1, half-maximal and maximal activities were observed at about 0.2 and 0.5 μM GTP, respectively. The increase in V_{max} due to PGE_1 was about 20 pmoles/mg per minute. At GTP concentrations higher than 0.5 μM, the stimulatory effect decreased slightly.

In order to compare the effects of PGE_1 on the low K_m GTPase activity and the adenylate cyclase activity, both assays were performed under almost identical conditions. App(NH)p, which was used in the GTPase assays in order to reduce the unspecific GTPase activity (23), was omitted in the adenylate cyclase assay and, additionally, the protein concentrations used for determination of adenylate cyclase activity were 3–4 times higher than those used in GTPase assays. With 0.3 μM GTP present, PGE_1 caused maximal inhibition of the adipocyte adenylate cyclase (70% inhibition) at about 0.1 μM, and half-maximal inhibition was observed at about 1 nM PGE_1 (Fig. 5). Similarly, maximal stimulation (almost 2-fold) of the low K_m GTPase was observed at about 0.1 μM PGE_1; half-maximal stimulation of the GTPase occurred at about 1 nM PGE_1. Thus, both inhibition of the adipocyte adenylate cyclase and stimulation of the low K_m GTPase exhibited almost identical concentration-response curves for PGE_1.

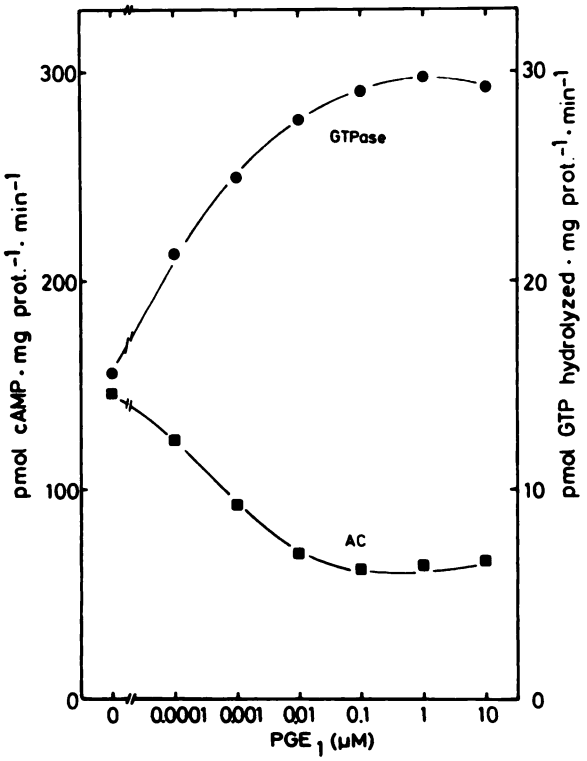

FIG. 5. *Influence of PGE_1 on the activities of low K_m GTPase and adenylate cyclase in hamster adipocyte membranes*

At increasing concentrations of PGE_1, low K_m GTPase activity (●, right ordinate; estimated as described in the legend in Fig. 2) and adenylate cyclase activity (■, left ordinate) were determined. The measurements of the activities of the two enzymes were performed under identical conditions with the exception that the adenylate cyclase assay medium contained no App(NH)p and a 3- to 4-fold higher protein concentration than the GTPase assay medium. GTP (0.3 μM), NaCl (100 mM), EDTA (0.1 mM), and cyclic AMP (0.1 mM) were present under each condition.

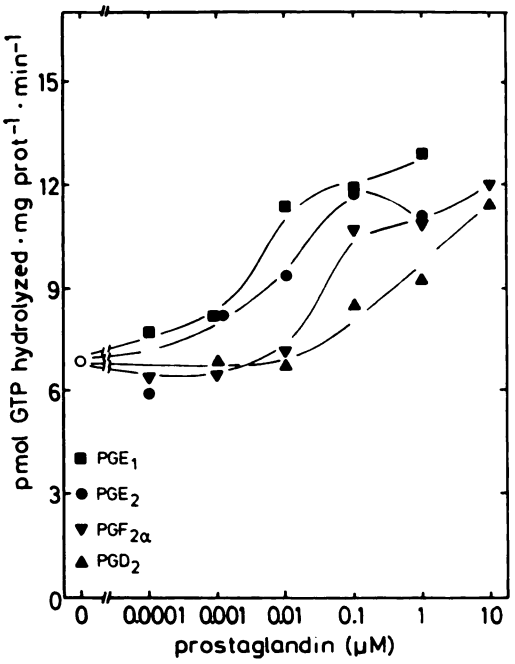

FIG. 6. *Effects of various prostaglandins on low K_m GTPase activity in adipocyte membranes*

Low K_m GTPase activity was determined in the absence and presence of increasing concentrations of PGE_1 (■), PGE_2 (●), $PGF_{2\alpha}$ (▼), and PGD_2 (▲) and estimated as described in the legend to Fig. 2. The GTP concentration was 0.1 μM.

In hamster adipocyte ghosts, inhibition of adenylate cyclase by various prostaglandins followed the potency order $PGE_1 \geq PGE_2 > PGF_{2\alpha} > PGD_2$, with similar maximal inhibition obtained with all the prostaglandins studied (20). Therefore, we questioned whether these prostaglandins would also stimulate the low K_m GTPase and whether the same rank order would be obtained. As shown in Fig. 6, all of the prostaglandins studied stimulated the low K_m GTPase, reaching the same level of activity. The potency order in stimulating the GTPase obtained was $PGE_1 \geq PGE_2 > PGF_{2\alpha} > PGD_2$.

Activation of a low K_m GTPase by hormones that stimulate adenylate cyclase has been described in a few tissues (23, 25–28). In hamster adipocyte ghosts, isoproterenol and ACTH, which stimulate adipocyte adenylate

TABLE 1

Influence of various inhibitory and stimulatory agents of the adenylate cyclase on low K_m GTPase activity in hamster adipocyte membrane preparations

Low K_m GTPase activity was determined in the absence and presence of the agents indicated and estimated as described in the legend to Fig. 2. GTP (0.3 μM), NaCl (100 mM), EDTA (0.1 mM), Ro 20-1724 (0.5 mM, instead of 3-isobutyl-1-methylxanthine), and adenosine deaminase (1.5 units/ml) were present under each condition. None of the hormonal factors had an effect on high K_m GTPase activity measured at 30 μM GTP. GTP hydrolyzed represents a typical experiment performed in triplicate, each value of which varied less than 5% of the mean.

	GTP hydrolyzed	GTPase activity
	pmoles/mg protein/min	*% control*
Control	13.5	100
PGE_1 (1 μM)	22.1	164
Nicotinic acid (30 μM)	20.6	153
3-Carboxy-5-methylpyrazole (30 μM)	19.6	145
N^6-Phenylisopropyladenosine (1 μM)	19.7	146
(−)-Isoproterenol (300 μM)	15.0	111
ACTH (10 μM)	14.1	104
KF (3 mM)	13.5	100

cyclase, had little effect on the low K_m GTPase activity. Under the assay conditions used, GTPase activity was increased by isoproterenol (300 μM) and ACTH (10 μM) by only about 10% (Table 1). Even in the additional presence of $CaCl_2$ (1 mM), the ACTH-induced stimulation was not enhanced. KF (3 mM), an unspecific activator of most adenylate cyclases (2), did not affect low K_m GTPase activity. In contrast, compounds which have been shown to inhibit hamster adipocyte adenylate cyclase, such as PGE_1 (1 μM), nicotinic acid (30 μM), 3-carboxy-5-methylpyrazole (29) (30 μM), and N^6-phenylisopropyladenosine (30) (1 μM), increased GTP hydrolysis by the low K_m GTPase by 45–65%. Epinephrine [300 μM; added in combination with 30 μM (±)-propranolol], which inhibits hamster adipocyte adenylate cyclase via *alpha*$_2$-adrenoceptors but to a smaller extent than PGE_1 (20), increased GTP hydrolysis by only about 20–30% (Table 2). The effects of PGE_1 and nicotinic acid (or epinephrine) were not additive. The adenylate cyclase stimulants, ACTH and KF, had no significant effect on the PGE_1-induced stimulation.

TABLE 2

Influence of PGE_1 in combination with various other compounds affecting adenylate cyclase activity on low K_m GTPase activity

Low K_m GTPase activity was determined in the absence and presence of the agents indicated and estimated as described in the legend to Figure 2. GTP (0.3 μM), NaCl (100 mM), and EDTA (0.1 mM) were present under each condition. (−)-Epinephrine was added in the presence of 30 μM (±)-propranolol. Values for GTP hydrolyzed are means of triplicate determinations.

	No PGE_1		PGE_1 (1 μM)	
	GTP hydrolyzed	GTPase activity	GTP hydrolyzed	GTPase activity
	pmoles/mg protein/min	*% control*	*pmoles/mg protein/min*	*% control*
Control	13.0	100	21.9	168
Nicotinic acid (30 μM)	22.3	172	22.0	169
(−)-Epinephrine (300 μM)	16.8	129	22.1	170
ACTH (10 μM)	14.7	113	22.7	175
KF (3 mM)	13.5	104	22.2	171

DISCUSSION

The existence of a low K_m GTPase, activated by adenylate cyclase stimulatory hormones, and a high K_m GTPase, unaffected by the hormone agonists, has been demonstrated in turkey erythrocyte membranes (23) and also in a few other tissues (25–28). The data presented in this communication show that, similarly, in hamster adipocyte membrane preparations at least two enzymes are responsible for GTP hydrolysis: a GTPase with high affinity for GTP (apparent K_m about 0.2 μM) and a GTPase with low affinity for GTP (apparent K_m about 180 μM). Similar to that in the above-mentioned tissues, the adipocyte high K_m GTPase was apparently not affected by any hormonal agent. On the other hand, in these membrane preparations, isoproterenol and ACTH caused only insignificant activation of the low K_m GTPase, although adenylate cyclase is potently stimulated by these hormones (20). This might be due to the high basal GTPase activity background as compared with that found in other tissues, e.g., turkey and frog erythrocyte membranes (23, 28). In contrast, hormonal agents known to inhibit hamster adipocyte adenylate cyclase, such as various prostaglandins (20), nicotinic acid (20), 3-carboxy-5-methylpyrazole (29), and N^6-phenylisopropyladenosine (30), caused large increases in low K_m GTPase activity.

There was a good correlation between data obtained in adenylate cyclase studies and in measurements of the low K_m GTPase activity with regard to actions of adenylate cyclase inhibitory agents. The concentrations of GTP required for half-maximal stimulation of GTPase (0.2 μM) and inhibition of adenylate cyclase (0.1 μM) (14) were almost identical. Furthermore, sodium ions have been shown to amplify hormone-induced inhibition of the adipocyte adenylate cyclase in membrane preparations, with a maximal effect at 100–200 mM (14). Similarly, sodium ions amplified the hormone-induced increase in GTPase activity with a maximal effect at 100–200 mM. Additionally, the concentration-response curves for adenylate cyclase inhibition and for low K_m GTPase stimulation were almost superimposable, as shown for PGE_1. There was also a good correlation between the potency order of various prostaglandins to inhibit adipocyte adenylate cyclase and to stimulate low K_m GTPase. Epinephrine, which via *alpha*$_2$-adrenoceptors inhibits hamster adipocyte adenylate cyclase but less effectively than PGE_1 or nicotinic acid (20), was also less effective on the low K_m GTPase.

We have recently observed (1, 31) that, in the presence of sodium, PGE_1 and nicotinic acid accelerated the turn-off reaction for isoproterenol-stimulated hamster adipocyte adenylate cyclase, whereas these inhibitory agents

not cause any delay in the adenylate cyclase turn-on reaction. The data presented in this report suggest that the acceleration of the turn-off reaction is due to the increased GTP hydrolysis at the regulatory site, by stimulation of a low K_m GTPase. Stimulation of a low K_m GTPase similar to that described in hamster adipocytes was observed in membrane preparations of human platelets; in these membranes, epinephrine via *alpha*$_2$-adrenoceptors inhibited adenylate cyclase and potently stimulated a low K_m GTPase (32). Thus, it is feasible that increased GTPase activity with concomitant increased inactivation of the adenylate cyclase is an essential mechanism of hormone-induced adenylate cyclase inhibition. Such a mechanism would also explain why adenylate cyclase inhibition by hormones is prevented or reversed by GTP analogues resistant to hydrolysis (10, 13, 15, 17–20), which have been shown to block low K_m GTPase in turkey erythrocyte membranes (33). Furthermore, an increased inactivation of the active adenylate cyclase by inhibitory hormones would also explain the frequent finding in intact cells that the inhibitory hormone-induced fall in cyclic AMP levels is most pronounced in the presence of a stimulatory hormone (10). On the basis of the present data, the active adenylate cyclase with GTP bound to the regulatory protein, the formation of which is promoted by stimulatory hormone-receptor interaction (4), appears to represent the substrate for the enzymatic step under control of the inhibitory hormones.

Although evidence is accumulated that increased GTP hydrolysis plays an essential role in negative hormonal control of adenylate cyclase activity, there are still large gaps in this suggested model which require filling. The main problem is determining how inhibitory hormones increase GTPase activity. Stimulatory hormones may indirectly increase GTP hydrolysis by promoting the binding of the substrate, GTP, to the regulatory protein (4). Inhibitory hormones may also promote the formation of a regulatory protein-adenylate cyclase complex. But this complex would have to be more susceptible to GTPase-dependent inactivation than that induced by stimulatory hormones. By this increased inactivation, the equilibrium of the adenylate cyclase would be shifted to the inactive state of the enzyme under the influence of inhibitory hormones. On the other hand, it is possible that the GTPase activation by inhibitory hormones is mediated by a regulatory protein which is not involved in hormone-induced stimulation of adenylate cyclase. Furthermore, it cannot be excluded that there are two different GTPases regulating adenylate cyclase activity, one involved in adenylate cyclase inhibition by hormones and another one apparently terminating hormone-induced stimulation; the latter one appears to be inhibited by cholera toxin (7) causing ADP-ribosylation of a component of the regulatory protein (8, 9). This assumption is supported by the finding that inhibitory hormones can decrease cholera toxin-activated adenylate cyclase activity as shown in human platelets (17, 19), rabbit myocardium (13), and neuroblastoma × glioma hybrid cells (34). The view that two GTPases are involved in the regulation of adenylate cyclase activity is further strengthened by the recent finding that *N*-ethylmaleimide can selectively uncouple *alpha*-adrenoceptor-mediated inhibition of adenylate cyclase and stimulation of low K_m GTPase in human platelets, whereas the alpha-adrenoceptor recognition site and the stimulatory effects of PGE_1 on platelet adenylate cyclase and GTPase were not significantly impaired (35). Purification of the inhibitory coupling component and reconstitution into defective systems will lead to an understanding of the functional differences of the GTP-binding and hydrolyzing components involved in stimulatory and inhibitory receptor-cyclase coupling.

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