Biochimica et Biophysica Acta, 445 (1976) 246—258

BBA 67868

HUMAN FAT CELL ADENYLATE CYCLASE

ENZYME CHARACTERIZATION AND GUANINE NUCLEOTIDE EFFECTS ON EPINEPHRINE RESPONSIVENESS IN CELL MEMBRANES

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(Received January 20th, 1976)

Summary

Human adenylate cyclase (ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1) has been studied in preparations of fat cell membranes ("ghosts"). As reported earlier, under ordinary assay conditions (1.0 mM ATP, 5 mM Mg^{2+}, 30°C, 10 min incubation) the enzyme was activated 6-fold by epinephrine in the presence of the GTP analog, 5'-guanylyl-imidodiphosphate [GMP-P(NH)P] (Cooper, B. et al. (1975) J. Clin. Invest. 56, 1350—1353). Basal activity was highest during the first 2 min of incubation then slowed and was linear for at least the next 18 min. Epinephrine, added alone, was often without effect, but sometimes maintained the initial high rate of basal activity. GMP-P(NH)P alone produced inhibition ("lag") of basal enzyme early in the incubation periods. Augmentation of epinephrine effect by GMP-P(NH)P, which also proceeded after a brief (2 min) lag period, was noted over a wide range of substrate (ATP) concentrations. GTP inhibited basal levels of the enzyme by about 50%. GTP also allowed expression of an epinephrine effect, but only in the sense that the hormone abolished the inhibition by GTP. Occasionally a slight stimulatory effect on epinephrine action was seen with GTP. At high Mg^{2+} concentration (>10 mM) or elevated temperatures (>30°C) GMP-P(NH)P alone activated the enzyme. Maximal activity of human fat cell adenylate cyclase was seen at 50 mM Mg^{2+}, 1.0 mM ATP, pH 8.2, and 37°C in the presence of 10^{-4} M GMP-P(NH)P; under these conditions addition of epinephrine did not further en-

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Abbreviations: GMP-P(NH)P, 5'-guanylyl-imidodiphosphate; cyclic AMP, 3',5'-adenosine monophosphate; XTP, xanthosine 5'-triphosphate.

hance activity. Human fat cell adenylate cyclase of adults was insensitive to ACTH and glucagon even in the presence of GMP-P(NH)P.

Introduction

We have recently described a nucleotide-dependent epinephrine sensitive adenylate cyclase (ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1) in human fat cell membranes [1]. Activation of the enzyme by epinephrine alone could not be shown to be statistically significant. The addition of the GTP analog, GMP-P(NH)P, together with epinephrine gave 5.7-fold activation; GMP-P(NH)P alone was generally without effect. This apparent absolute requirement of GMP-P(NH)P for expression of epinephrine sensitive activity differed from that of rat fat cell adenylate cyclase, where 6-fold activation was noted with epinephrine alone [2].

Nucleotide effects on the catecholamine activation of adenylate cyclase have been previously demonstrated in a variety of systems. In the frog erythrocyte and in canine heart, GMP-P(NH)P enhanced activation of catecholamine-sensitive adenylate cyclase [3,4] while in the turkey erythrocyte several purine nucleotides [ITP, XTP, GTP, GMP-P(NH)P] stimulated isoproterenol-sensitive adenylate cyclase [5,6]. Moreover, GTP enhanced epinephrine-sensitive adenylate cyclase in rat liver plasma membranes [7]. In fat cell membranes, however, guanine nucleotide effects have previously been studied only in the rat, where GTP usually inhibited and GMP-P(NH)P enhanced epinephrine activation [8—11]. However, recent studies have shown that at a high concentration of Mg^{2+} both basal and hormone stimulated adenylate cyclase are enhanced by GTP [12].

In this report we present an extension of our studies on human fat cell adenylate cyclase and its nucleotide dependence for hormone activation. Although a number of similarities between the human and rat enzyme are apparent, especially the sensitivity of nucleotide effects to Mg^{2+} concentration and temperature, the human enzyme has been found to behave quite differently from that of the rat in terms of the range of hormone sensitivity and in its striking dependence on a guanine nucleotide for epinephrine activation.

Methods

Preparation of tissue. Samples of subcutaneous fat (5—15 g) were obtained from the anterior abdominal wall of adult male and female patients undergoing elective abdominal surgery. Patients excluded from this study were subjects with severe obesity, diabetes mellitus, or poor nutritional status. After obtaining informed consent samples were taken after an overnight fast with the patients under general anesthesia. The tissue was placed immediately in 37°C isotonic NaCl, transported to the laboratory within 5 min, and dissected free of connective tissue. Isolated fat cells and fat cell membranes ("ghosts") were prepared as previously described [1]. Ghosts were assayed for adenylate cyclase activity within 15 min after preparation.

Assay of adenylate cyclase. Enzyme activity was measured by the method of

Salomon et al. [13]. The standard assay mixture contained $1.5 \cdot 10^6$ dpm [α-^{32}P]ATP (New England Nuclear; 10—30 Ci/mM); 1.0 mM ATP (Sigma); 25 mM Tris · HCl, pH 7.4; 5.0 mM $MgCl_2$; 2 mM cyclic AMP (Sigma); ^{3}H-labelled cyclic AMP, 10^4 dpm (New England Nuclear), 0.1% albumin (Armour, fraction V); 10 mM theophylline (Sigma), and an ATP regenerating solution consisting of 11 mM creatine phosphate and 1 mg/ml creatine kinase (Sigma). Reactions were initiated by the addition of 20 μl of suspended membranes (5—20 μg) and were terminated by the addition of a sodium lauryl sulfate solution containing excess ATP and cyclic AMP. Approximately 10^4-dpm ^{14}C-labelled cyclic AMP (Schwarz/Mann) was added and the reaction vessels heated for 5 min in a boiling water bath. Unless otherwise stated incubations were for 10 min at 30°C. The uses of ^{3}H-labelled cyclic AMP and ^{14}C-labelled cyclic AMP for monitoring phosphodiesterase activity and column recovery have already been described [1]. All data were expressed as nmol cyclic AMP/10 min per mg protein. Determination of ghost protein was by the method of Lowry et al. [14]. All assays were performed in duplicate; the points shown are the means of these values. Individual experiments used ghosts from fat cells of a single patient. Hormones and activators included L-epinephrine bitartrate (Sigma), sodium fluoride (Fisher), GMP-P(NH)P (ICN Pharmaceuticals), GTP (Sigma), and porcine ACTH (Calbiochem, grade B). Crystalline porcine glucagon was a gift from Eli Lilly and Co.

Results

Time course

The time course of human fat cell adenylate cyclase in the basal state and in the presence of epinephrine and fluoride is shown in Fig. 1A. Under standard assay conditions basal activity was initially high (first 2 min; thereafter activity was slower but remained linear for at least the next 18 min. (Fig. 1A; 1B). The marked enzyme activation by fluoride (11-fold over basal at 10 min) was in contrast to the apparent and slight stimulatory effect of epinephrine. This effect of epinephrine appeared, in fact, to represent merely a preservation of the initial relatively high rate of activity of the basal enzyme (Fig. 1A and 1B). Fig. 1B (see inset) illustrates that the addition of GMP-P(NH)P produced an inhibitory lag phase early in the incubation period. Because of this initial inhibition, GMP-P(NH)P alone did not usually enhance basal levels at 10 min of incubation *. When epinephrine was added along with GMP-P(NH)P, activation of the enzyme occurred with a lag phase which was perhaps slightly shorter than that produced by GMP-P(NH)P alone. These results are in contrast to previous studies of this enzyme in the rat, where GMP-P(NH)P produced less initial inhibition of shorter duration and uniformly stimulated the enzyme at 10 min under similar assay conditions [4,10—12,15]. Rodbell [12] noted elimination of the GMP-P(NH)P induced lag phase during ACTH activation of rat fat cell adenylate cyclase. We did not observe such a striking effect by epinephrine on the GMP-P(NH)P induced lag phase with the human enzyme. This type of interaction between nucleotide and hormone has been interpreted as providing strong evidence for the existence of various enzyme transition states [12,16].

* The effect of GMP-P(NH)P alone has been shown to be variable but statistically not significantly different from basal activity under standard assay conditions [1].

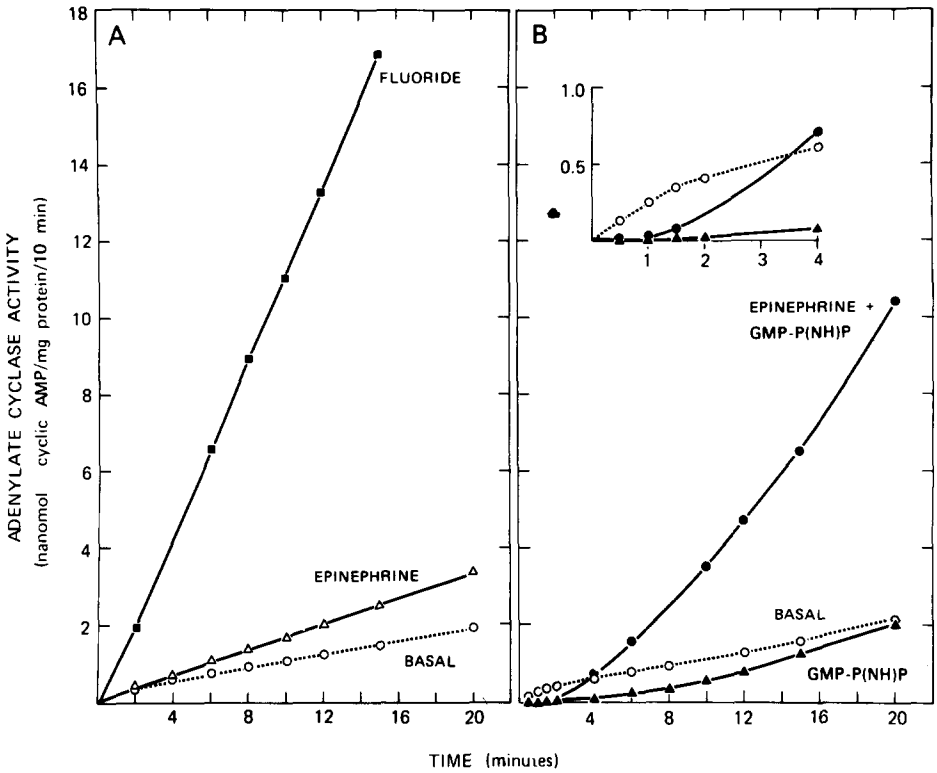

Fig. 1. Time course of human fat cell adenylate cyclase: effects of epinephrine and fluoride (A) and of GMP-P(NH)P alone and in the presence of epinephrine (B). Fat cell ghosts were incubated at 30°C under standard assay conditions as described under Methods. GMP-P(NH)P, 10^{-4} M; epinephrine, 10^{-3} M; and fluoride, $5 \cdot 10^{-3}$ M. Inset demonstrates lag effect of GMP-P(NH)P and the possible shortening of the GMP-P(NH)P-induced lag period by epinephrine. In most experiments epinephrine alone produced no appreciable effect, but when an effect was seen, as in this experiment, a concentration of 10^{-3} M was generally necessary. In the presence of GMP-P(NH)P lower concentrations of epinephrine were maximally effective (see Results).

Effects of Mg^{2+} concentration

Fig. 2 shows the effects of Mg^{2+} concentration on human fat cell adenylate cyclase at 1 mM ATP. Basal levels were Mg^{2+} concentration-dependent up to the highest Mg^{2+} concentration tested (50 mM). At concentrations of Mg^{2+} above 10 mM the addition of GMP-P(NH)P enhanced enzyme activity. The GMP-P(NH)P-dependent epinephrine sensitive enzyme was most strongly activated over the range 1—10 mM Mg^{2+}, but was still further augmented between 10 and 50 mM.

This absolute Mg^{2+} requirement for human fat cell adenylate cyclase is similar to most other adenylate cyclase studies [17], although a few recently described enzymes appear to be Mn^{2+}-dependent [18]. We have not studied the effect of Mn^{2+} on the human enzyme. The true substrate for adenylate cyclase appears to be Mg · ATP. The concentration of Mg^{2+} also determines the concentration in the reaction mixture of free ATP, which, in contrast to Mg · ATP, mimics the activating effects of guanine nucleotides at the nucleotide regulatory site [12,19].

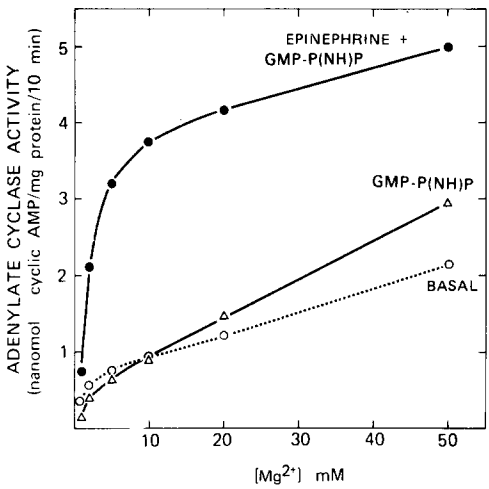

Fig. 2. Effects of Mg^{2+} concentration on human fat cell adenylate cyclase for basal, GMP-P(NH)P, and epinephrine plus GMP-P(NH)P-stimulated enzyme. Fat cell ghosts were incubated at 30°C under standard assay conditions except for Mg^{2+} concentration, as described under Methods. GMP-P(NH)P, 10^{-4} M; epinephrine, 10^{-3} M; and fluoride, $5 \cdot 10^{-3}$ M.

Effects of temperature

Human fat cell adenylate cyclase was not temperature dependent in the basal state or in the presence of epinephrine alone (Fig. 3). However, the enzyme became temperature dependent when GMP-P(NH)P was added. While GMP-P-

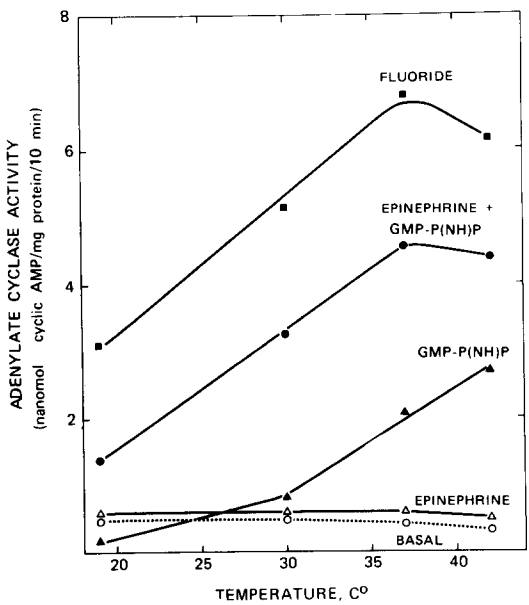

Fig. 3. Effects of temperature on human fat cell adenylate cyclase in the presence of epinephrine, GMP-P(NH)P, and fluoride. Fat cell ghosts were incubated under standard assay conditions as described under Methods. GMP-P(NH)P, 10^{-4} M; epinephrine, 10^{-3} M; and fluoride, $5 \cdot 10^{-3}$ M.

(NH)P alone inhibited the enzyme at 18°C, the nucleotide activated steeply and linearly between 30°C and 42°C. The augmenting effect of GMP-P(NH)P on epinephrine-sensitive enzyme was also temperature dependent, increasing linearly between 18°C and 37°C. This curve paralleled the temperature dependence for fluoride activation. Assays at temperatures up to 30°C (5 mM Mg^{2+}) generally showed GMP-(PNH)P-dependent epinephrine activation without an effect by GMP-P(NH)P alone. However, 30°C was a critical inflection point for temperature activation. This phenomenon may also account for the variable effects of GMP-P(NH)P alone on the enzyme at this temperature, if individual preparations vary slightly in their temperature dependence and the exact point of inflection.

Effects of ATP concentration

Basal levels of human fat cell adenylate cyclase were maximal between 1 and 2 mM ATP (Fig. 4). Epinephrine alone usually failed significantly to enhance the enzyme over a wide range of ATP concentrations while the effect of GMP-P(NH)P alone (2-fold activation in this experiment) was maximal at 1.0 mM ATP. In the presence of GMP-P(NH)P epinephrine stimulation increased 2-fold between 0.1 and 1.0 mM ATP, then gradually decreased with additional substrate. The substrate curve for fluoride activation was similar with maximal activity at 2.0 mM ATP. In contrast to the human enzyme where the addition of epinephrine alone was no more substrate dependent than that of basal levels,

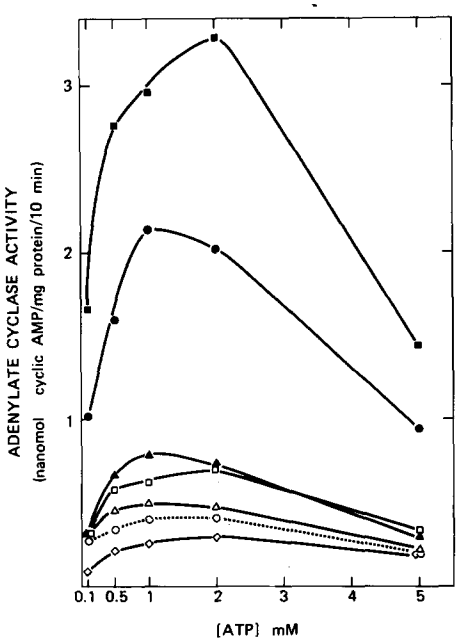

Fig. 4. Effects of substrate concentration (ATP) on human fat cell adenylate cyclase in the presence of epinephrine, GMP-P(NH)P, GTP, and fluoride. Fat cell ghosts were incubated at 30°C under standard assay conditions except for ATP concentration, as described under Methods. Additions include none (○———○), GMP-P(NH)P, 10^{-4} M (▲———▲); GTP, 10^{-4} M (◇———◇); epinephrine, 10^{-3} M (△———△); fluoride, $5 \cdot 10^{-3}$ M (■———■); GTP, 10^{-4} M + epinephrine, 10^{-3} M (□———□); GMP-P(NH)P, 10^{-4} M + epinephrine, 10^{-3} M (●———●).

in rat fat cell adenylate cyclase epinephrine activation in the absence of nucleotide increased relative to basal levels at higher (>0.3 mM) ATP concentrations [9]. These results in the rat are comparable to the optimum ATP concentration for human fat cell adenylate cyclase in the presence of both epinephrine and GMP-P(NH)P.

The nucleotide dependence of hormone activation of various animal adenylate cyclases is frequently a function of substrate concentration (ATP or its analog, 5'-adenylyl-imidodiphosphate). Ebert and Schwabe [10] have recently demonstrated a requirement for GMP-P(NH)P in the norepinephrine activation of rat fat cell adenylate cyclase at very low ATP concentration (0.03 mM). Others also using low substrate concentrations have demonstrated an absolute requirement for GTP in the adenylate cyclase stimulation by epinephrine or glucagon with rat liver [7,20] and pancreatic β cells [21,22]; by prostaglandins with human platelets [23]; and by luteinizing hormone-releasing factor with the rat anterior pituitary [24]. There also appears to be a requirement for GTP in the activation of adenylate cyclase in human thyroid membranes by a prostaglandin (PGE_2) even at moderate (1.0 mM) ATP concentrations [25]. This thyroid system and our presently reported epinephrine-sensitive human fat cell adenylate cyclase are unusual in requiring a guanine nucleotide for hormone activation over a wide range of ATP concentrations under ordinary assay conditions (i.e., 5—10 mM Mg^{2+}).

Effects of GTP

GTP alone was inhibitory for human fat cell adenylate cyclase under our assay conditions (Fig. 4). Although in this experiment GTP activated the epinephrine-sensitive human enzyme at all ATP concentrations tested, the effect was small compared to the marked stimulation by GMP-P(NH)P in the pres-

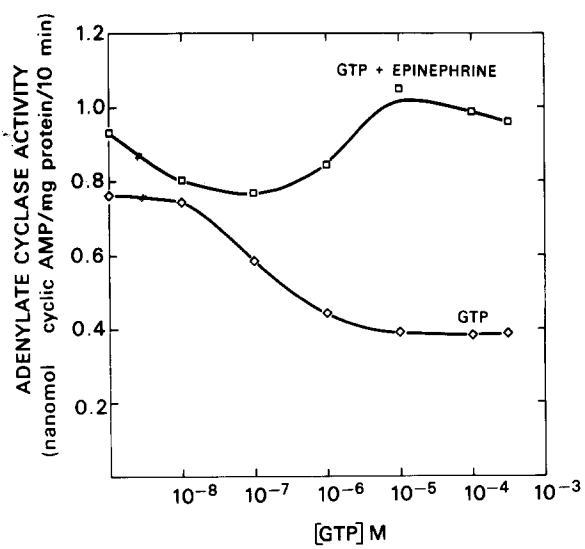

Fig. 5. Effects of GTP concentration on human fat cell adenylate cyclase in the presence and absence of epinephrine. Fat cell ghosts were incubated under standard assay conditions at 30°C as described under Methods. Epinephrine, 10^{-3} M.

ence of epinephrine. Fig. 5 illustrates that this effect of GTP on epinephrine activation was not always stimulatory; in this experiment no activation was apparent even at high concentrations of GTP. The addition of epinephrine did, however, prevent inhibition by GTP (Fig. 5), suggesting that some interaction between GTP and epinephrine was occurring. Others have previously reported that GTP also inhibited both basal and epinephrine stimulated rat fat cell adenylate cyclase under comparable conditions [8,9].

Effects of pH

Between pH 6.5 and 8.4 the activity of human fat cell adenylate cyclase increased only slightly both in the basal state and in the presence of epinephrine or GMP-P(NH)P (Fig. 6). These findings were in contrast to the marked pH dependence of the enzyme noted in the presence of both GMP-P(NH)P and epinephrine; between pH 6.5 and 7.8 enzyme activation increased 3.4-fold. Fluoride activation was also pH-dependent. Rodbell [12] has shown that the stimulation of rat fat cell adenylate cyclase by GMP-P(NH)P is maximal at pH 8.1—8.5. He postulates that higher pH reduces the concentration of protonated substrate which is inhibitory for the enzyme.

Effects of GMP-P(NH)P at 50 mM Mg^{2+} and 37°C

Because of the activation by GMP-P(NH)P alone at high Mg^{2+} concentrations

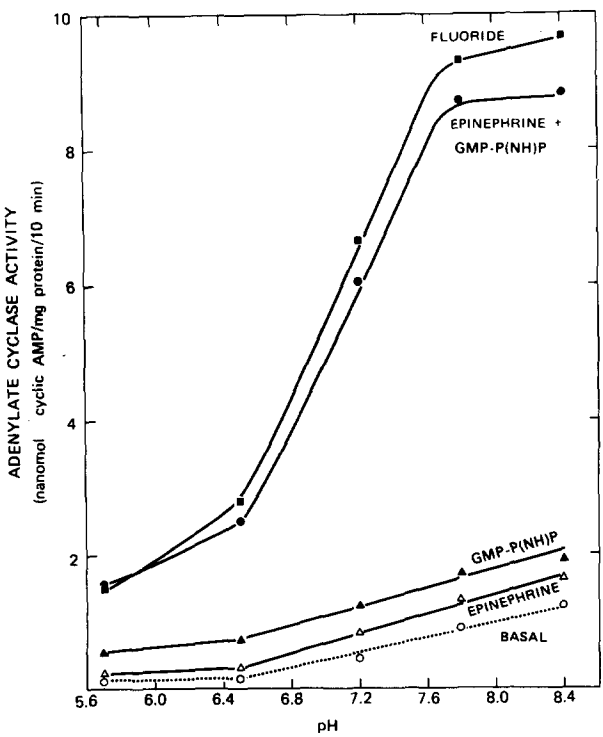

Fig. 6. Effects of pH on human fat cell adenylate cyclase in the presence of GMP-P(NH)P, epinephrine, and fluoride. Fat cell ghosts were incubated at 30°C under standard assay conditions as described under Methods. GMP-P(NH)P, 10^{-4} M; epinephrine, 10^{-3} M; and fluoride, $5 \cdot 10^{-3}$ M.

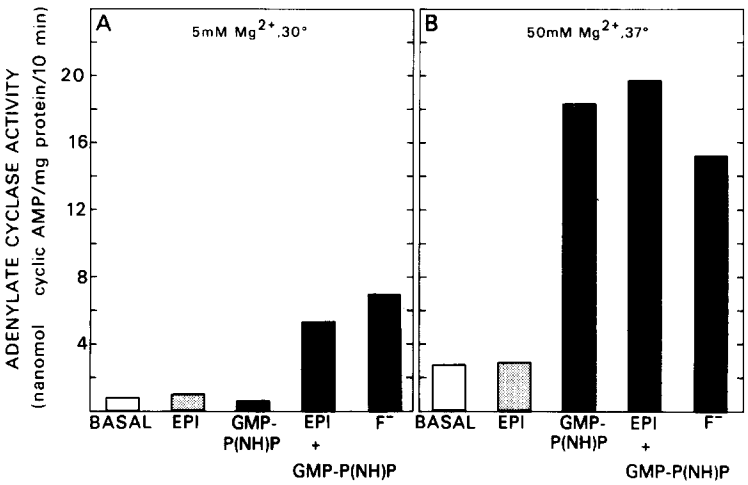

Fig. 7. Comparison of GMP-P(NH)P, epinephrine, and fluoride activation of human fat cell adenylate cyclase at 5 mM Mg^{2+}, 30°C (A) to activation at 50 mM Mg^{2+}, 37°C (B). ATP, 1.0 mM; pH 8.2; 10 min incubations. GMP-P(NH)P, 10^{-4} M; epinephrine, 10^{-3} M; and fluoride, $5 \cdot 10^{-3}$ M.

or elevated temperature (Figs. 2 and 3), the enzyme was studied at 37°C and 50 mM Mg^{2+}. Fig. 7 compares these results to studies on the same enzyme preparation under standard assay conditions (5 mM Mg^{2+}, 30°C). While basal levels were enhanced at 37°C and 50 mM Mg^{2+}, stimulation over basal by epinephrine was not observed (Fig. 7). However, while GMP-P(NH)P alone inhibited basal levels at 30°C and 5 mM Mg^{2+} (Fig. 7A), a 6.5-fold activation was produced by GMP-P(NH)P at 37°C and 50 mM Mg^{2+} (Fig. 7B). The further addition of epinephrine along with GMP-P(NH)P only slightly enhanced enzyme activity (Fig. 7B). In other experiments even small increases in activity were not uniformly observed with epinephrine under these assay conditions. At 37°C and 50 mM Mg^{2+} fluoride activation was considerably less than stimulation by GMP-P(NH)P. Rodbell et al. [15] have previously demonstrated maximal activity of rat fat cell adenylate cyclase at 37°C, pH 8.5, 1.0 mM ATP, 50 mM Mg^{2+}, in the presence of 10^{-4} M GMP-P(NH)P. The addition of hormone (epinephrine) increased enzyme activity only slightly (15%) under these conditions.

Effects of glucagon and ACTH

Human fat cell adenylate cyclase was not stimulated by ACTH or glucagon (Table I). In this particular experiment the enzyme was activated by epinephrine alone. However, as already noted, the response to epinephrine was variable, often absent, and could not be shown to be statistically significant (see Discussion). The addition of GMP-P(NH)P slightly increased basal activity in this experiment and allowed the expression of epinephrine sensitivity. We have previously shown that in the presence of GMP-P(NH)P epinephrine activation is first noted at 10^{-6} M hormone with near maximal effect at 10^{-4} M. A wide range of concentrations of glucagon and ACTH did not activate the enzyme even in the presence of GMP-P(NH)P. The restricted hormone sensitivity of human fat cell adenylate cyclase is in contrast to the stimulation of rat fat cell adenylate cyclase by epinephrine (6-fold), glucagon (4.5-fold) and ACTH (4-

TABLE I

EFFECTS OF VARIOUS HORMONES ON HUMAN FAT CELL ADENYLATE CYCLASE IN THE PRESENCE AND ABSENCE OF GMP-P(NH)P

Fat cell ghosts were assayed under incubation conditions as described under Methods (pH 8.2).

Additions	Adenylate cyclase activity (nmol cyclic AMP/10 min per mg protein)	
	Without GMP-P (NH)P	With GMP-P (NH)P (10^{-4} M)
None	0.46	0.86
Epinephrine (10^{-3} M)	0.77	2.91
Epinephrine (10^{-4} M)	0.72	3.34
Epinephrine (10^{-5} M)	0.62	2.79
ACTH (10^{-4} M)	0.49	0.82
ACTH (10^{-5} M)	0.44	0.79
Glucagon (10^{-5} M)	0.48	0.81
Glucagon (10^{-6} M)	0.44	0.79
Fluoride ($5 \cdot 10^{-3}$ M)	2.99	—

fold) reported by us and others under comparable assay conditions [2,11]. With the rat enzyme the addition of GMP-P(NH)P increased both basal activity and hormone responsiveness [9,11,12,15].

Discussion

We have recently reported an apparent absolute requirement for a GTP analog, GMP-P(NH)P, for the reproducible activation by epinephrine of human fat cell adenylate cyclase [1]. Guanine nucleotides have been shown to exert a variety of regulatory effects on hormone-sensitive adenylate cyclases in a number of animal tissues [15,26]. These nucleotides generally augment hormone action, but inhibition has been noted in a few cases [8]. GMP-P(NH)P has been particularly effective in enhancing hormone-sensitive adenylate cyclase. This analog's stimulatory effects are greater than those of GTP even though the latter appears to have greater affinity for the enzyme's regulatory binding sites [6]. The mechanism of nucleotide interaction with the enzyme appears complex, but it has been suggested that the nucleotides are involved in the allosteric regulation of a number of adenylate cyclase systems [12,15,16].

In the present studies we have observed human fat cell adenylate cyclase and its GMP-P(NH)P-dependent epinephrine sensitivity under a variety of experimental conditions. Enzyme activity was greatly influenced by substrate concentration, pH, temperature, Mg^{2+} concentration, and the length of incubation. These parameters influenced basal levels, fluoride stimulation, and the effects of GMP-P(NH)P by itself and in the presence of epinephrine. Although we reported earlier that GMP-P(NH)P alone was without effect on the enzyme, it is now clear that this finding was the result of the conditions (5 mM Mg^{2+}; 30°C) under which the original studies were performed [1]. At higher temperatures (>30°C) or greater concentration of Mg^{2+} (50 mM) GMP-P(NH)P does indeed exert a stimulatory effect of its own, as has been shown in other systems. Furthermore, our earlier statement that GTP, unlike GMP-P(NH)P, is without any enhancing effect on epinephrine activation must also be modified. GTP is regu-

larly inhibitory of basal activity, but in its presence epinephrine exerts a stimulatory effect. However, the effect is usually only enough to overcome the GTP inhibition; stimulation beyond the original (GTP absent) basal level is not regularly observed. Only occasionally can a small, clearly stimulatory effect be seen (e.g., Fig. 4). Under none of the conditions so far tested has GTP had the major stimulatory effect on epinephrine-sensitive human fat cell adenylate cyclase that can be regularly demonstrated with GMP-P(NH)P.

We have found a number of similarities between the human enzyme and the previously described rat fat cell adenylate cyclase. In both species basal levels and fluoride activation showed similar patterns of sensitivity with respect to pH, temperature, and the concentrations of Mg^{2+} and substrate. However, under certain conditions the human and rat enzymes differed in their responses to hormones and to guanine nucleotides. GMP-P(NH)P uniformly stimulated rat fat cell adenylate cyclase [3,9—12] while with the human enzyme only high Mg^{2+} concentration (>10 mM) or elevated temperature (>30°C) allowed significant analog enhancement of the enzyme (Figs. 2 and 3). Human fat cell adenylate cyclase was insensitive to epinephrine alone while the rat enzyme was very responsive to this hormone [2]. Nonetheless, in the presence of GMP-P(NH)P the human and rat enzymes behaved similarly in their response to epinephrine and in regard to their dependence on Mg^{2+} concentration, pH, temperature, and substrate concentration.

The only prior reports of catecholamine stimulation of human fat cell adenylate cyclase of which we were aware at the time of this work were those of Burns and Langley [27] and Burns et al. [28] who noted 20% stimulation of adenylate cyclase by epinephrine and 2-fold activation over basal by isoproterenol. Their results were reported only in preliminary form, and statistical evaluation of the catecholamine activation was not presented. Effects of guanine nucleotides were not reported. After submission of our present manuscript, those authors published their results in more detail and have shown statistically significant increases of adenylate cyclase activity with epinephrine alone (1.6-fold) and isoproterenol (2.3-fold). The ability of these workers to demonstrate relatively small but statistically significant effects of catecholamines alone and our own earlier failure to do so [1] may now be reconciled. We have re-examined our previously published data of basal activity versus epinephrine alone (8 experiments). In 4 experiments appreciable activation did occur with epinephrine; in 3 experiments values with epinephrine were slightly higher than without; in 1 experiment no effect whatever was seen. The ratio of our mean value with epinephrine was 1.4 times that without the hormone, and the difference was not significant by unpaired t test [1]. When we now apply the paired t test as did Burns and Langley [29], the difference becomes significant ($P < 0.01$). Thus, our results and those of the latter workers are in fact similar, although we never saw significant stimulation at their levels of epinephrine (10^{-5} M) and needed a much higher concentration (10^{-3} M) to observe any effect in the absence of added GMP-P(NH)P. Other experimental variables were different in their work as opposed to our own (temperature; substrate concentration). These or other experimental conditions may yet prove to be important determinants of optimal catecholamine activation of human fat cell adenylate cyclase and of the effects of guanine nucleotides. However, our own obser-

vation is that the apparent effect of epinephrine, in the absence of guanine nucleotide, seems to be one of preserving initial high basal activity (Fig. 1). One may certainly wonder, therefore, about the real significance of the small increment at a single point in time (10 min) seen with epinephrine alone. Unfortunately, Burns and Langley [29] have not reported on the time course of the epinephrine effect they observed.

The demonstration of a nucleotide-dependent epinephrine sensitive human fat cell adenylate cyclase is consistent with the well described lipolytic effects of catecholamines in human fat cells [30—33]. In the rat catecholamines promote lipolysis by a mechanism which appears to be mediated by cyclic AMP via activation of adenylate cyclase [34]. Our results suggest a similar role for adenylate cyclase in mediating catecholamine-sensitive lipolysis in man. In addition, it has previously been shown in human fat cells that catecholamines produce a prompt rise in intracellular cyclic AMP prior to its lipolytic effects [35,36], a finding which further suggests a role for cyclic AMP (and adenylate cyclase) in the mediation of catecholamine-sensitive lipolysis in human fat.

From the present studies it seems clear that the previously demonstrated lack of lipolytic responsiveness of human fat cells to ACTH and glucagon [31, 35] is related to the insensitivity of human fat cell adenylate cyclase to these hormones. To our knowledge ACTH and glucagon have not previously been studied for their effects on human fat cell adenylate cyclase. In contrast to its effect on epinephrine sensitive adenylate cyclase the addition of GMP-P(NH)P did not allow the demonstration of any enzyme activation by ACTH or glucagon (Table I).

Our results with the human enzyme are in contrast to the well demonstrated sensitivity of rat fat cell adenylate cyclase to a variety of hormones, including ACTH and glucagon [2]. However, we have recently shown both ACTH and glucagon-sensitive fat cell adenylate cyclases in the rat to be age-dependent [11]. While glucagon activated adenylate cyclase of the 1-month rat about 4.5-fold relative to basal, the mature 6-month rat was completely insensitive to this hormone. The loss of ACTH activation with age in rat fat cell adenylate cyclase was less pronounced, i.e., 4-fold stimulation in the 1-month rat vs 2-fold in the senescent (24-month) animal. In contrast, the relative stimulation (stimulated activity vs. basal) of this enzyme by epinephrine was independent of age in the rat. Since to date only adult human fat has been used in our own experiments and in previous studies by others of lipolysis, the possibility remains that ACTH- or glucagon-sensitive adenylate cyclases may yet be demonstrable in fat cells of the human fetus or child.

Acknowledgments

We thank the surgical staff of Baltimore City Hospitals for their assistance in obtaining the fat specimens used in these studies and Mrs. Peggy Zimmerman for help in the preparation of this manuscript.

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