

# Adenylate Cyclase of Human Fat Cells

## EXPRESSION OF EPINEPHRINE-SENSITIVE ACTIVATION REVEALED BY 5'-GUANYLYL-IMIDODIPHOSPHATE

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**ABSTRACT** Although catecholamines stimulate lipolysis in human fat cells, activation by epinephrine of adenylate cyclase in human fat cell membranes is not readily observed. The possible role of guanine nucleotides in this reaction has now been examined with human material. Fat cell ghosts were prepared from subcutaneous fat obtained from patients undergoing elective surgery. Adenylate cyclase was assayed with [ $\alpha$ - $^{32}$ P]ATP as substrate. Fluoride ion stimulated the enzyme 8.3-fold relative to basal levels, but epinephrine activation of cyclase was not statistically significant. GTP did not allow expression of an epinephrine effect. However, the addition of the GTP analogue, 5'-guanylyl-imidodiphosphate [GMP-P(NH)P], along with epinephrine produced 5.7-fold activation of the enzyme ( $P < 0.001$ ). GMP-P(NH)P alone was without stimulatory effect. Comparable augmentation by GMP-P(NH)P of adenylate cyclase activity was seen with isoproterenol, norepinephrine, and epinephrine. Propranolol blocked catecholamine-GMP-P(NH)P stimulation of the enzyme, suggesting that the nucleotide-dependent activation of catecholamine-sensitive adenylate cyclase is mediated by  $\beta$ -receptors. GMP-P(NH)P may prove useful in allowing in vitro demonstration of additional hormone-sensitive adenylate cyclase systems.

### INTRODUCTION

The catecholamines promote lipolysis in fat cells of the rat by a mechanism which appears to be mediated by cyclic AMP via activation of adenylate cyclase (1). Catecholamines are also lipolytic for fat cells of man

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(2-5), but direct effects of these hormones on human adenylate cyclase have not been clearly documented. Preliminary reports have suggested only slight activation by epinephrine of human fat cell adenylate cyclase (6, 7). When we were unable to demonstrate significant catecholamine activation of cyclase in human fat cell ghosts isolated by methods which regularly produced for us highly responsive preparations in the rat, we decided to investigate the possible role of guanine nucleotides as regulators of this response in the human tissue. These compounds have previously been demonstrated to exert a variety of effects on animal cyclases (8-11). This report demonstrates an augmenting effect of the GTP analogue, 5'-guanylyl-imidodiphosphate [GMP-P(NH)P],<sup>1</sup> on human fat cell catecholamine-sensitive adenylate cyclase.

### METHODS

**Source of fat 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. Each patient gave informed consent for removal of the fat specimen. Severely obese patients and those with diabetes were excluded. Samples were obtained at the beginning of surgery with the patients under general anesthesia after an overnight fast. Data on lipolysis in human fat cells suggest that general anesthesia does not inhibit the metabolism of adipose tissue (2).

**Preparation of fat cell ghosts.** Fat samples were immediately placed in 37°C isotonic NaCl (4, 5) and transported to the laboratory within 5 min. After samples were dissected free of connective tissue, isolated fat cells were prepared by treatment with collagenase (Worthington Biochemical Corp., Freehold, N. J., type I) as described by Rodbell (12). Human cells proved to be more fragile than those from rats. Accordingly, the speed of the metabolic incubator did not exceed 100 oscillations/min. Some lots of collagenase were unsuitable for the preparation of human cells, giving

<sup>1</sup>Abbreviations used in this paper: cAMP, 3',5'-cyclic AMP; GMP-P(NH)P, 5'-guanylyl-imidodiphosphate.

insufficient yields because of inadequate digestion of fat or lysis of the cells. In the experiments reported here, the same lot of collagenase was used throughout. Plasma membranes or "ghosts" were prepared from the cells by a minor modification of the method of Harwood and Rodbell (13) in which 1 mM dithiothreitol replaces mercaptoethanol in the lysing and suspending media. Yields of ghost protein (1 mg/10 g whole fat) were less than half those obtained from rat epididymal fat in our laboratory. All ghosts were assayed for cyclase activity within 15 min after preparation.

**Assay of adenylate cyclase.** Enzyme activity was measured by the method of Salomon et al. (14). This is a labeled substrate assay using [ $\alpha$ - $^{32}$ P]ATP as substrate with measurement of [ $^{32}$ P]3',5'-cyclic AMP (cAMP) product. The standard assay mixture contained  $1.5 \times 10^8$  dpm [ $\alpha$ - $^{32}$ P]-ATP (New England Nuclear, Boston, Mass.), 10–30 Ci/mM; 1.0 mM ATP (Sigma Chemical Co., St. Louis, Mo.), 25 mM Tris-HCl (pH 7.4); 5.0 mM MgCl<sub>2</sub>; 2 mM cAMP (Sigma); 0.1% albumin (Pentex Biochemical, Kankakee, Ill., Fraction V); 10 mM theophylline (Sigma) and an ATP regenerating solution consisting of 20 mM creatine phosphate and 1 mg/ml creatine kinase (Sigma). [ $^3$ H]-cAMP (New England Nuclear) was included in the incubation medium to monitor possible loss or destruction of the [ $^{32}$ P]cAMP product by phosphodiesterase. Incubation mixtures contained a final volume of 50  $\mu$ l. Reactions were initiated by addition of 20  $\mu$ l of suspended ghosts (5–20  $\mu$ g) and continued for 10 min at 30°C. Enzyme activity was linear within this range of protein concentrations and time. Reactions were terminated by the addition of sodium lauryl sulfate containing excess carrier ATP and cAMP, followed by heating for 5 min in a boiling water bath. Approximately  $10^4$  dpm of [ $^{14}$ C]cAMP (Schwarz/Mann Div., Orangeburg, N. Y.), was added immediately after the stopping solution to monitor column losses. The recoveries for [ $^3$ H] and [ $^{14}$ C]cAMP were very nearly the same in all experiments, indicating absence of appreciable phosphodiesterase activity under our experimental conditions. All data were expressed as nanomoles cAMP per milligram ghost protein per 10 minutes. Determination of ghost protein was by the method of Lowry et al. (15).

Hormones and activators included L-epinephrine bitartrate, L-isoproterenol bitartrate, L-norepinephrine bitartrate, and L-phenylephrine hydrochloride (Sigma); propranolol hydrochloride (Ayerst Laboratories, New York); GMP-P(NH)P (ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio), and sodium fluoride (Fisher Scientific Co., Pittsburgh, Pa.). Each experiment involved assaying ghosts for enzyme activity from fat cells prepared from a single patient. Statistical analysis was by Student's *t* test for unpaired samples.

## RESULTS

Fig. 1 illustrates the nucleotide dependence of epinephrine-sensitive human fat cell adenylate cyclase. No significant increase in adenylate cyclase activity over basal levels was apparent in the presence of either epinephrine or GMP-P(NH)P. However, the addition of both GMP-P(NH)P and epinephrine activated the enzyme 5.7-fold over basal levels ( $P < 0.001$ ). This stimulatory effect compares with the 8.3-fold enhancement above basal activity noted in the presence of fluoride ( $P < 0.001$ ). While the permissive effect of GMP-P(NH)P on epinephrine-sensitive cyclase was noted in

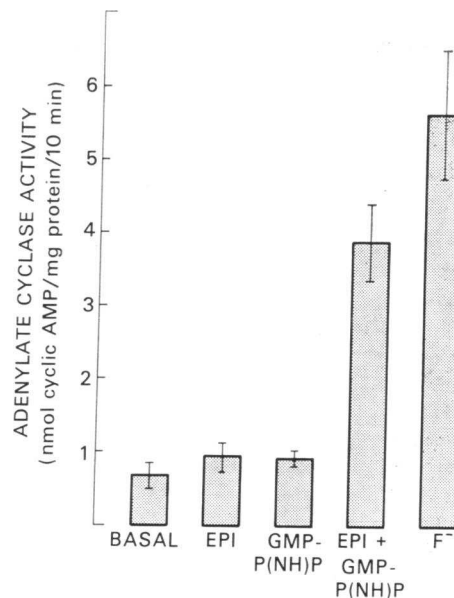


FIGURE 1 Effect of epinephrine (Epi,  $10^{-3}$  M), fluoride ( $F^-$ ,  $5 \times 10^{-3}$  M), and GMP-P(NH)P ( $10^{-4}$  M) on human fat cell adenylate cyclase. Results are mean  $\pm$  SEM for eight experiments.

all eight experiments, the effects of GMP-P(NH)P or epinephrine separately on the enzyme was less predictable. In no experiment was the stimulation by epinephrine alone greater than 2-fold over basal levels, and in several samples no effect was observed. The addition of GMP-P(NH)P alone in two experiments was mildly inhibitory and in two samples basal levels were doubled; no effect was noted in the remaining four experiments.

The effect of GMP-P(NH)P on the epinephrine dose-response curve is shown in Fig. 2 A. In this experiment the presence of epinephrine failed to stimulate

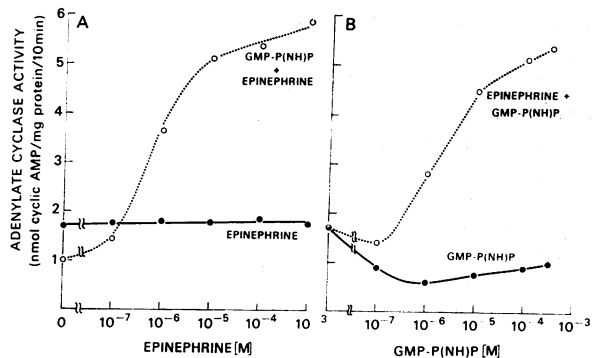


FIGURE 2 A. Effect of GMP-P(NH)P ( $10^{-4}$  M) on epinephrine dose-response curve. B. Effect of GMP-P(NH)P concentration on the response to epinephrine ( $10^{-3}$  M).

TABLE I  
Effect of Adrenergic Agonists and Propranolol on Human Fat Cell Adenylate Cyclase

Addition*	Adenylate cyclase activity	
	Without GMP-P(NH)P	With GMP-P(NH)P
	<i>nmol cAMP/mg ghost protein per 10 min</i>	
None	0.25	0.70
Isoproterenol	0.43	2.50
Epinephrine	0.43	2.70
Norepinephrine	0.32	2.57
Phenylephrine	0.23	1.14
Propranolol	0.20	0.55
Isoproterenol + propranolol	0.20	0.55
Epinephrine + propranolol	0.23	0.59
Norepinephrine + propranolol	0.23	0.68

\* Concentrations of additions were  $10^{-4}$  M except for propranolol ( $10^{-3}$  M).

the enzyme at any concentration tested; the addition of GMP-P(NH)P alone was slightly inhibitory. However, in the presence of the analogue enzyme activation was evident at  $10^{-6}$  M epinephrine with a near maximal effect at  $10^{-4}$  M. Fig. 2 B shows the effect of GMP-P(NH)P concentration on the response to epinephrine. In this instance stimulation of epinephrine-sensitive cyclase was first noted at  $10^{-6}$  M GMP-P(NH)P with the maximal effect at  $10^{-4}$  M. The fat sample used for the studies in Fig. 2 was obtained at splenectomy from a patient on short-term high-dose prednisone therapy given for idiopathic thrombocytopenic purpura, and this experiment is the only one reported involving a patient receiving any medications.

The effect of other adrenergic agonists on enzyme activity in human fat cell ghosts is shown in Table I. Isoproterenol and norepinephrine, like epinephrine, significantly enhanced enzyme activity over basal levels only in the presence of GMP-P(NH)P. The action of phenylephrine, a predominantly  $\alpha$ -adrenergic agonist, was also enhanced but not as markedly as with the  $\beta$ -adrenergic agonists. Propranolol, a  $\beta$ -adrenergic antagonist, was able to block completely the stimulatory effects of the catecholamines on the enzyme. These results suggest that the nucleotide-activated, catecholamine-sensitive cyclase of the human fat cell is dependent on  $\beta$ -receptors in the cell membrane. GMP-P(NH)P activated catecholamine-sensitive adenylate cyclase in rat fat cells has also been shown recently to be blocked by propranolol (10).

## DISCUSSION

A role for  $\beta$ -adrenergic receptors and cAMP in catecholamine and isoproterenol stimulated lipolysis of hu-

man fat cells has been clearly demonstrated (16, 17). However, the only prior reports of catecholamine stimulation of human fat cell adenylate cyclase are by Burns and Langley (6) and by Burns et al. (7). These authors noted 20% stimulation of adenylate cyclase by epinephrine while isoproterenol activation was greater (2-fold over basal). Their results were reported in preliminary form and statistical evaluation of the catecholamine activation was not presented. The effects of guanine nucleotides were not reported.

In our own studies the stimulation of human fat cell adenylate cyclase by epinephrine was not significant in the absence of GMP-P(NH)P. In contrast, almost 6-fold stimulation of the enzyme by epinephrine has been regularly seen in rat fat cell ghosts assayed in our laboratory (18). Others have earlier reported similar results under comparable assay conditions (19). The addition of GMP-P(NH)P to the human enzyme does not alter basal levels but results in a 5.7-fold enhancement of the enzyme activity in the presence of epinephrine (see Fig. 1). These results are in contrast to those seen with the enzyme of the rat. In that system GMP-P(NH)P not only enhances catecholamine sensitivity but by itself regularly stimulates basal levels in the absence of hormones (8, 10). We have also evaluated the parent nucleotide, GTP, for its effect on human fat cell cyclase. As noted with the rat enzyme (8, 10), this compound inhibits basal levels. However, with GTP we have not observed the augmenting action for epinephrine on the human enzyme noted with GMP-P(NH)P.

In addition to the stimulation of catecholamine-sensitive adenylate cyclase by GMP-P(NH)P demonstrated in rat fat cell membranes (8, 10) and now in human fat, similar effects have been shown in canine myocardium, and frog and turkey erythrocytes (10, 11). This phenomenon is not restricted to catecholamine-sensitive enzyme; enhancement of adenylate cyclase activity by GMP-P(NH)P or other guanine nucleotides has been demonstrated with glucagon, thyrotrophin, prostaglandins, vasopressin, ACTH, secretin, and other hormones in various tissues (20, 21). In a few instances and under highly specific conditions (e.g. low substrate concentrations), others have shown an absolute nucleotide requirement for hormone activation of adenylate cyclase. However, the GMP-P(NH)P dependence of epinephrine activation of human fat cell cyclase reported here is apparently not the result of such special circumstances; the requirement is manifest under all assay conditions thus far tested, including lower and higher ATP concentrations.

The augmenting effect of GMP-P(NH)P which allows demonstration of a catecholamine-sensitive adenylate cyclase suggests a role for guanine nucleotides in

the action of human fat cell cyclase. Moreover, the action of GMP-P(NH)P on human fat cell adenylate cyclase raises the interesting possibility that this agent may permit demonstration of hormone-sensitive adenylate cyclases in additional human or animal tissues which otherwise appear insensitive.

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#### REFERENCES

1. Robison, G. A., R. W. Butcher, and E. W. Sutherland. 1971. Lipolysis in adipose tissue. In *Cyclic AMP*. G. A. Robison, R. W. Butcher, and E. W. Sutherland, editors. Academic Press, Inc., New York. 285-316.
2. Galton, D. J., and G. A. Bray. 1967. Studies on lipolysis in human adipose cells. *J. Clin. Invest.* **46**: 621-629.
3. Burns, T. W., and P. Langley. 1968. Observations on lipolysis with isolated adipose tissue cells. *J. Lab. Clin. Med.* **72**: 813-823.
4. Moskowitz, J., and J. N. Fain. 1969. Hormonal regulation of lipolysis and phosphorylase activity in human fat cells. *J. Clin. Invest.* **48**: 1802-1808.
5. Goldrick, R. B., and G. M. McLoughlin. 1970. Lipolysis and lipogenesis from glucose in human fat cells of different sizes. Effects of insulin, epinephrine and theophylline. *J. Clin. Invest.* **49**: 1213-1223.
6. Burns, T. W., and P. E. Langley. 1971. Further observations on the role of the adenylyl cyclase-cyclic AMP (cAMP) system in human lipolysis. *J. Lab. Clin. Med.* **78**: 989. (Abstr.)
7. Burns, T. W., P. E. Langley, and G. A. Robison. 1972. Studies on the role of cyclic AMP in human lipolysis. *Adv. Cyclic Nucleotide Res.* **1**: 63-85.
8. Harwood, J. P., H. Löw, and M. Rodbell. 1973. Stimulatory and inhibitory effects of guanyl nucleotides on fat cell adenylate cyclase. *J. Biol. Chem.* **248**: 6239-6245.
9. Londos, C., Y. Salomon, M. C. Lin, J. P. Harwood, M. Schramm, J. Wolff, and M. Rodbell. 1974. 5'-guanylylimidodiphosphate, a potent activator of adenylate cyclase systems in eukaryotic cells. *Proc. Natl. Acad. Sci.* **71**: 3087-3090.
10. Lefkowitz, R. J. 1974. Stimulation of catecholamine-sensitive adenylate cyclase by 5'-guanylyl-imidodiphosphate. *J. Biol. Chem.* **249**: 6119-6124.
11. Spiegel, A. M., and G. D. Aurbach. 1974. Binding of 5'-guanylyl-imidodiphosphate to turkey erythrocyte membranes and effects on  $\beta$ -adrenergic-activated adenylate cyclase. *J. Biol. Chem.* **249**: 7630-7636.
12. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239**: 375-380.
13. Harwood, J. P., and M. Rodbell. 1973. Inhibition by fluoride ion of hormonal activation of fat cell adenylate cyclase. *J. Biol. Chem.* **248**: 4901-4904.
14. Salomon, Y., C. Londos, and M. Rodbell. 1974. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* **58**: 541-548.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
16. Burns, T. W., and P. E. Langley. 1970. Lipolysis by human adipose tissue: The role of cyclic 3',5'-adenosine monophosphate and adrenergic receptor sites. *J. Lab. Clin. Med.* **75**: 983-997.
17. Burns, T. W., P. E. Langley, and G. A. Robison. 1971. Adrenergic receptors and cyclic AMP in the regulation of human adipose tissue lipolysis. *Annals N. Y. Acad. Sci.* **185**: 115-128.
18. Cooper, B., and R. I. Gregerman. 1976. Relation of age and cell size to hormone stimulated rat adipose adenylate cyclase. *J. Clin. Invest.* In press.
19. Birnbaumer, L., and M. Rodbell. 1969. Adenylyl cyclase in fat cells. II. Hormone receptors. *J. Biol. Chem.* **244**: 3477-3482.
20. Rodbell, M., M. C. Lin, Y. Salomon, C. Londos, J. P. Harwood, B. R. Martin, M. Rendell, and M. Berman. 1974. The role of adenine and guanine nucleotides in the activity and response of adenylate cyclase systems to hormones: evidence for multi-site transition states. *Acta Endocrinol.* **77** (Suppl. 191): 11-37.
21. Birnbaumer, L., and P. C. Yang. 1974. Studies on receptor-mediated activation of adenylyl cyclases. III. Regulation by purine nucleotides of the activation of adenylyl cyclases from target organs for prostaglandins, luteinizing hormone, neuro hypophyseal hormones and catecholamines. Tissue- and hormone-dependent variations. *J. Biol. Chem.* **249**: 7867-7873.