Activation of adenosine 3′,5′-monophosphate-dependent protein kinase and its relationship to cyclic AMP and lipolysis in hamster adipose tissue

Richard J. Schimmel, Christine A. Buhlinger, and Rosalind Serio

Department of Physiology, College of Medicine and Dentistry of New Jersey, New Jersey School of Osteopathic Medicine, Piscataway, NJ 08854

Abstract The interrelationships among cAMP-dependent protein kinase activity, lipolysis, and cellular concentrations of cAMP were investigated in hamster epididymal adipose tissue. Isoproterenol, norepinephrine, and theophylline increased the protein kinase activity assayed in tissue extracts with no added cAMP, but not in the presence of added cyclic nucleotide. The maximum rate of lipolysis was associated with a nearly three-fold increase in cAMP levels and a protein kinase activity ratio of 0.8 (the ratio of activity assayed without cAMP to that assayed with cAMP). Rates of lipolysis less than maximum were associated with lesser degrees of protein kinase activity and lower levels of cAMP. The relatively pure α-adrenergic agent phenylephrine partially suppressed the isoproterenol-stimulated protein kinase activity, lipolysis, and cAMP levels. Conversely, the α-adrenergic blocking agent phentolamine increased the activity of protein kinase and cAMP levels in adipose tissues exposed to norepinephrine. These data are consistent with the primary role for cAMP and its dependent protein kinase in control of lipolysis in adipose tissue. Moreover, our data are consistent with the view that the antilipolytic action of α-adrenergic agents is mediated by a decrease in activity of protein kinase, caused by a decrease in cellular cAMP concentrations.—**Schimmel, R. J., C. A. Buhlinger, and R. Serio.** Activation of adenosine 3′,5′-monophosphate-dependent protein kinase and its relationship to cyclic AMP and lipolysis in hamster adipose tissue. *J. Lipid Res.* 1980. **21:** 250–256.

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The mobilization of triglyceride stored in adipose tissue cells occurs by its hydrolysis to free fatty acids and glycerol. The rate limiting enzyme catalyzing this hydrolysis is known as "hormone sensitive lipase" (1). An impressive body of evidence can be marshalled suggesting that hormones increase the activity of this key enzyme by a sequence of events involving increased intracellular accumulation of cAMP, activation of a soluble protein kinase enzyme, and increased phosphorylation of lipase (1–3). The evidence supporting this mechanism has been gathered primarily from studies on rat adipose tissue. Unfortunately, firmly establishing the relationship between the level of cAMP and the rate of lipolysis in rat adipose tissue has proved difficult. This is due in large measure to two characteristics of rat adipose tissue. In rat adipose tissue, cyclic AMP levels increase only transiently following stimulation with epinephrine, or another suitable lipolytic hormone, while lipolysis proceeds at a constant rate (4–8). Secondly, lipolysis may be markedly increased by concentrations of epinephrine that do not significantly increase cyclic AMP levels but which cause a 50% increase in activity of the protein kinase enzyme (2, 9). The interpretation most often given to these findings is that lipolysis is regulated over an extremely narrow range of cellular cyclic AMP levels (2, 9).

In contrast to rat adipose tissue, the cAMP response of hamster fat cells to lipolytic hormones is much more extended and of greater magnitude; levels of cAMP in hamster fat cells exposed to catecholamines may remain elevated for 2 hr of incubation (10). These properties of hamster adipose tissue suggest that it may be a more useful experimental system in which to study the mechanisms underlying hormonal activation of lipolysis. Moreover, hamster adipose tissue responds to α-adrenergic stimulation with a partial suppression of cAMP levels and lipolysis (10–14) but the role of the cAMP-protein kinase system in the inhibition of lipolysis is uncertain. The relationships among cAMP, protein kinase, and lipolysis in hamster fat have not been studied, and it seemed that our understanding of the mechanism of action of α-adrenergic agents as well as our understanding of the regulation of lipolysis in this tissue would benefit from such a study.

METHODS AND MATERIALS

Normal golden male hamsters (Charles River, Lakeview, NJ) weighing between 100 and 120 g were used.

They were maintained on Purina Lab Chow and tap water ad libitum for at least 1 week before use. Following cervical dislocation, both epididymal fat bodies were rapidly excised. Segments of the fat bodies were cut free hand with a scissors and were combined to give sample weights of 200 to 300 mg. Tissue samples were incubated at 37°C with shaking in 2.0 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 1% bovine serum albumin (Sigma, Fraction V) under an atmosphere of 95% O_2 and 5% CO_2 (v/v) in the presence or absence of various drugs and hormones. Unless otherwise specified, the incubation time was 15 min.

Following incubation, the tissues were homogenized in two volumes (w/v) of cold 10 mM EDTA, 10 mM potassium phosphate, 0.5 mM 1-methyl-3-isobutyl xanthine, and 0.5 M NaCl at pH 6.5 using a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenates were centrifuged at 12,000 *g* for 10 min at 4°C and the infranatant fraction collected for assay of protein kinase.

Protein kinase activity of adipose tissue extracts was determined using the method described by Corbin, Soderling, and Park (15). A 0.050 ml aliquot of a reaction mixture composed of 10 mg/ml histone (Worthington Biochemical Corp., Freehold, NJ), 17 mM potassium phosphate, 18 mM magnesium acetate, and 0.33 mM [^{32}P] ATP (approximately 100 cpm/pmol; supplied by New England Nuclear Corp., Boston, MA) at pH 6.5 and 0.005 ml of 400 mM sodium fluoride was added to 0.020 ml of the infranatant fraction. Activity was assayed in the absence and in the presence of exogenous cAMP (1.33 μM) and expressed as the ratio of these activities (−cAMP/+cAMP). Assays were run at 30°C for 10 min. The rate of phosphorylation of histone was found to be constant for up to 20 min of incubation and directly proportional to the amount of tissue extract present in the assay.

The reaction was terminated by transferring 0.50 ml of the mixture onto 2-cm^2 filter paper squares (Whatman Inc., Clifton, NJ, ET31) which were immediately dropped into a beaker containing 10% trichloroacetic acid. The acid solution was changed a total of four times at approximately 15 min intervals. The filters remained in the 10% trichloroacetic acid solution overnight. The following day, the filters were washed successively in 95% ethanol and in diethyl ether for 5 min each and then allowed to dry in air. No means of stirring was used during any step in the washing sequence. After drying, the filter papers were placed in toluene-based scintillation fluid for counting.

Cyclic AMP content of the tissue homogenate was determined on 0.100 ml aliquots of a 1 to 10 (v/v) dilution in 50% acetic acid (17) of the adipose tissue extract using the protein binding assay described by Gilman (18) as modified by Mashiter et al. (19). The amount of glycerol released into the incubation media was measured fluorometrically using a modification (7) of the enzymatic method described by Wieland (20).

TABLE 1. Effect of sodium chloride on protein kinase activity ratio

	Protein Kinase Activity Ratio (−cAMP/+cAMP)
Initial	0.61 ± .07
Gel filtration on Sephadex G-25; eluted with homogenization buffer without salt	0.18 ± .02
Gel filtration on Sephadex G-25; eluted with homogenization buffer; assayed in presence of 0.5 M NaCl	0.21 ± .02
Gel filtration on Sephadex G-25; eluted with homogenization buffer containing 0.5 M NaCl	0.52 ± .06

Segments of hamster adipose tissue were incubated with 10 μM isoproterenol for 15 min and were then homogenized in two volumes (w/v) of a solution of 10 mM EDTA, 10 mM potassium phosphate, 0.5 mM 1-methyl-3-isobutyl xanthine and 0.5 M NaCl, pH 6.5. Following centrifugation at 12,000 *g* for 10 min, aliquots of the infranatant fraction were removed for assay of protein kinase (initial) or were applied to columns (0.9 × 8 cm) of Sephadex G-25 at 4°C. One column was equilibrated and eluted with homogenization buffer, the other column with homogenization buffer containing 0.5 M NaCl. The protein kinase activity ratio was determined on the protein peak. Each point represents the mean ± SE of three separate experiments.

The composition of the buffer mixture used for homogenization of the tissues and the protein kinase assay procedure were described by Corbin et al. (15) to be suitable for use with a Type II protein kinase. We have determined that the protein kinase enzyme of hamster fat behaves in a fashion similar to that described for the kinase present in rat adipose tissue. Thus, the protein kinase activity ratio was greatly reduced after removal of cAMP by gel filtration through 0.9 × 8 cm columns of G-25 Sephadex (**Table 1**). Inclusion of 0.5 M NaCl in the buffer used to elute the column partially preserved the protein kinase activity ratio suggesting, therefore, that the presence of the NaCl inhibited the reassociation of the subunits after removal of cAMP. Addition of NaCl to the kinase eluted in the absence of NaCl failed to cause a significant increase in the activity ratio. In addition, chromatography on DEAE cellulose showed that the hamster adipose tissue enzyme migrated as a single peak of cAMP-dependent activity, eluting at approximately 0.21 M NaCl (**Fig. 1**). By comparison, the enzyme from rat adipose tissue similarly chromatographs as a single peak eluting at 0.15 to 0.25 M NaCl whereas the type I kinase from rat heart elutes at 0.05 to 0.10 M NaCl (16). These data indicate that the

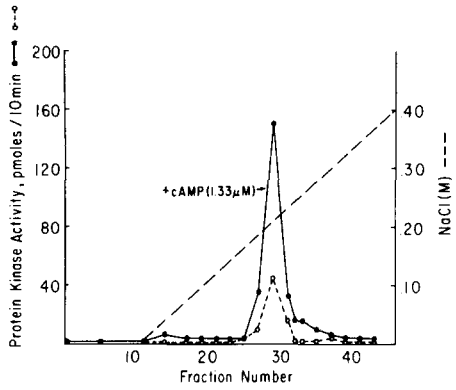

Fig. 1. DEAE-cellulose chromatography of protein kinase in crude extracts of adipose tissue. Adipose tissue was homogenized in a solution of 5 mM Tris-1 mM EDTA (pH 7.5). A DEAE-cellulose column (0.9 × 3.5 cm) was equilibrated with the same buffer and then 3 ml of adipose tissue extract was added. The column was washed with 30 ml of buffer and a linear gradient of NaCl (0 to 400 mM) was started. Approximately 50 fractions of 3 ml each were collected and assayed for protein kinase in the absence or presence of 1.33 μM cAMP.

protein kinase in hamster white adipose tissue should be characterized as a Type II enzyme (15).

RESULTS

Fig. 2 shows the effect of graded concentrations of isoproterenol on lipolysis, accumulation of cAMP, and activity of protein kinase. The lowest concentration of isoproterenol that produced a consistent increase in activity of protein kinase was 1 μM. At this concentration, the rate of lipolysis was increased

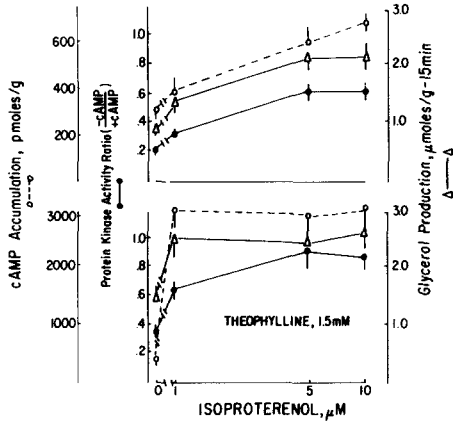

Fig. 2. Activation of protein kinase, lipolysis, and accumulation of cAMP by isoproterenol in the absence (upper panel) and presence (lower panel) of theophylline. Segments of epididymal adipose tissue were incubated in 2 ml of buffer containing 1% bovine serum albumin and various amounts of isoproterenol with or without 1.5 mM theophylline for 15 min. Each point represents the mean ± SE of at least eight observations.

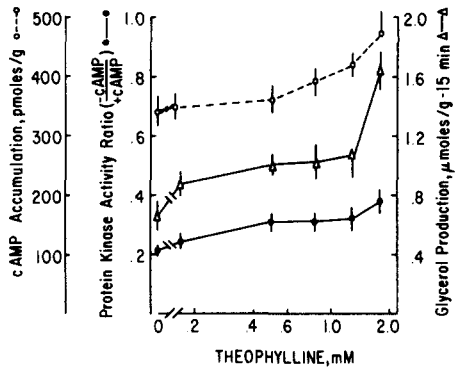

Fig. 3. Activation of protein kinase, lipolysis, and accumulation of cAMP by theophylline. Segments of epididymal adipose tissue were incubated in 2 ml of buffer containing 1% bovine serum albumin and various amounts of theophylline for 15 min. Each point represents the mean ± SE of four observations.

nearly twofold and the concentration of cAMP and the degree of activation of protein kinase were both slightly but significantly increased. Higher concentrations of isoproterenol were associated with greater levels of cAMP, rates of lipolysis, and protein kinase activity. When norepinephrine was used, similar data were obtained except that norepinephrine was slightly less effective at increasing lipolysis, accumulation of cAMP, and protein kinase activity ratio than was isoproterenol (not presented). Total protein kinase activity assayed in the presence of 1.33 μm cAMP was not influenced by isoproterenol or norepinephrine or indeed by any other treatment employed in the present investigation (data not presented).

When theophylline was present, isoproterenol elicited very large increases in cAMP, but increased lipolysis and the activity of protein kinase to levels only slightly greater than that seen in the absence of theophylline. Theophylline by itself (**Fig. 3**) increased lipolysis, the degree of activation of the protein kinase, and tissue cAMP levels, although not to the same levels as did isoproterenol.

An important characteristic of hamster adipose tissue that is not shared by rat adipose tissue is an α-adrenergic inhibition of lipolysis and partial suppression of cAMP levels (10–14). **Fig. 4** presents the effects of the α-adrenergic blocking agent phentolamine on the activity of protein kinase in tissues exposed to norepinephrine (5.0 μM). Norepinephrine was selected for this study because it possesses both α- and β-adrenergic activity. In response to norepinephrine, the activity ratio of protein kinase increased from 0.21 to 0.38, cAMP levels from 232 to 447 pmol/g, and lipolysis from 0.59 to 1.67 μmol glycerol/g-15 min. These values are all slightly lower than those seen in the presence of 5 μM isoproterenol (Fig.

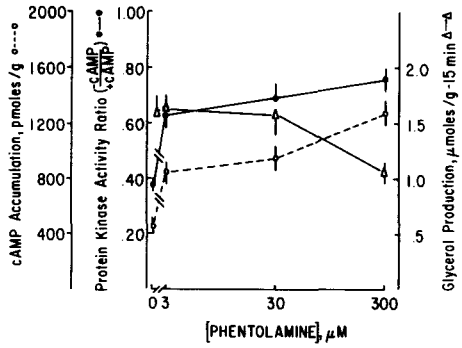

Fig. 4. Effects of phentolamine on activity of protein kinase, rates of lipolysis, and levels of cAMP in tissues exposed to norepinephrine. Segments of epididymal adipose tissue were incubated in 2 ml of buffer containing 1% bovine serum albumin for 15 min. Norepinephrine was present at all points plotted at a concentration of 5 μM. Both norepinephrine and phentolamine were added at zero time. Basal values (not shown) in the absence of norepinephrine and pentolamine were: protein kinase activity ratio, 0.21 ± 0.01, glycerol production, 0.59 ± 0.03 μmol/g-15 min, cAMP, 232 ± 47 pmol/g. Each point represents the mean ± SE of six observations.

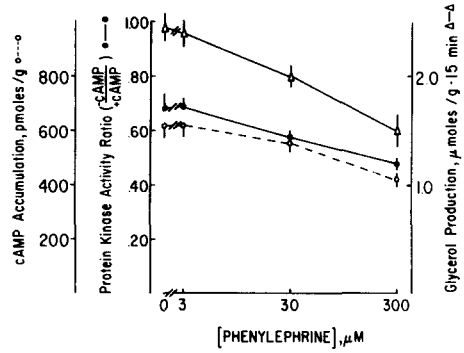

Fig. 5. Effects of phenylephrine on activity of protein kinase, rates of lipolysis, and levels of cAMP in tissues stimulated with isoproterenol. Segments of epididymal adipose tissue were incubated in 2 ml of buffer containing 1% bovine serum albumin for 15 min. Isoproterenol was present at all points plotted at a concentration of 5 μM. Both isoproterenol and phenylephrine were added at zero time. Basal values (not shown) in the absence of isoproterenol or phenylephrine were as follows: protein kinase activity ratio, 0.24 ± 0.02; glycerol production, 0.44 ± 0.04 μmol/g-15 min; cAMP, 305 ± 19 pmol/g. Each point represents the mean ± SE of eight observations.

2). That the lower effectiveness of norepinephrine results from the α-adrenergic activity of the catecholamine is suggested by the finding that the α-adrenergic blocking drug phentolamine produced large additional increases in the activity ratio of protein kinase and in the concentration of cAMP. The rate of lipolysis, however, was unaffected by phentolamine at concentrations up to 30 μM; higher concentrations of the α-adrenergic blocker inhibited lipolysis. Control experiments (data not presented) indicated that phentolamine by itself had no effect upon cAMP, protein kinase or on lipolysis.

Fig. 5 shows that the selective α-adrenergic agent phenylephrine produced a partial inhibition of lipolysis, accumulation of cAMP, and the degree of activation of the protein kinase enzyme in tissues that had been stimulated by isoproterenol (5.0 μM).

In **Fig. 6** we have taken the results within each different incubation condition (depicted in Figs. 2, 3 and 5) and plotted the relationship between glycerol production and the protein kinase activity ratio. This figure does not include values obtained from the experiment using phentolamine but does include the data obtained from the study of the effects of graded concentrations of norepinephrine on lipolysis, cAMP levels, and activity of protein kinase, which was not depicted. There appeared to be a good correlation between the protein kinase activity ratio and the rates of glycerol production for activity ratios between 0.20 and 0.80. Higher activity ratios were occasionally observed, particularly in response to the combination of isoproterenol and theophylline, but these were not associated with greater rates of lipolysis.

In **Fig. 7** we have constructed a similar plot, using results from each different incubation condition, relating tissue levels of cAMP to the activity ratio of protein kinase. There appeared to be good correlation between tissue cAMP and the protein kinase activity ratio for cAMP levels from 300 to 800 pmol/g. cAMP levels of 800 pmol/g were associated with a protein kinase activity ratio of 0.80, which was in turn associ-

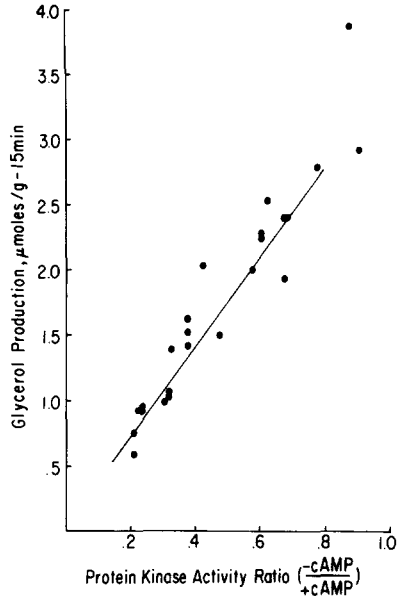

Fig. 6. The relationship between glycerol production and protein kinase activity ratio in hamster adipose tissue. The data in this figure were taken from Figs. 2, 3, and 5. Hence, each point represents a mean response obtained from a single incubation condition, having an incubation time of 15 min.

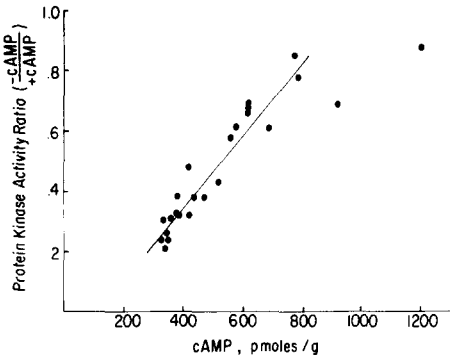

Fig. 7. The relationship between the protein kinase activity ratio and the level of cAMP in hamster adipose tissue. The data in this figure were taken from Figs. 2, 3, and 5. Hence, each point represents a mean response obtained from a single incubation condition, having an incubation time of 15 min.

ated with the maximum rate of lipolysis. Higher cAMP levels, as could be produced in tissues exposed to the combination of isoproterenol and theophylline, were associated with protein kinase activity ratios greater than 0.80 but these were not associated with higher rates of lipolysis.

DISCUSSION

The present study was undertaken to evaluate the hypothesis (1–3, 9) that hormone-stimulated lipolysis in adipose tissue is mediated by a cAMP-dependent activation of protein kinase. Hamster adipose tissue was selected for this study because cAMP responses to appropriate lipolytic hormones are greater than are seen in rat adipose tissue and are not evanescent (10); and because such a study afforded a means of studying the role of protein kinase in action of α-adrenergic agents (13). In the main, the results obtained provide strong support for the view, advanced on the basis of experiments on rat adipose tissue (1–3, 9), that changes in the activation state of the protein kinase are brought about by changes in cellular cAMP levels and further, that changes in the rate of lipolysis are mediated by changes in the activity of the protein kinase enzyme.

The activity ratio of the protein kinase enzyme in unstimulated hamster fat was found to be approximately 0.20. In several experiments it was measured to be as low as 0.15 and occasionally as high as 0.25. The basal activity ratio in rat adipose tissue has similarly been found to be between 0.15 and 0.25 (7, 9, 15, 21, 22). The similar basal protein kinase activity ratio in rat and hamster fat is surprising given the higher basal cAMP levels in the hamster tissue (10, 12, 14). One possible explanation would be the presence of greater amounts of the heat-stable protein kinase inhibitor (23) in hamster fat. The basal protein kinase activity ratio in isolated rat adipocytes has been reported to be somewhat higher (20, 21) than that measured in tissue segments and the protein kinase activity ratio in hamster adipocytes is higher than in hamster fat pads.[1]

In contrast to the similar protein kinase activity ratios in unstimulated rat and hamster adipose tissue, the activity ratios associated with activated lipolysis were markedly different in the two tissues. In hamster adipose tissue, maximum lipolysis was associated with a protein kinase activity ratio of 0.80 and a nearly three-fold increase in cellular cAMP levels. In contrast, lipolysis in rat white adipose tissue was reported to be maximal when only about 40% of the protein kinase enzyme was activated (9), a degree of enzyme activation associated with a 50% increase in cAMP levels (9). In brown fat from rats, lipolysis is maximally activated at a protein kinase activity ratio of 0.50 (22). It would appear, therefore, that lipolysis in hamster adipose tissue is regulated over a wider range of cAMP levels and activity of the protein kinase enzyme than is lipolysis occurring in rat adipose tissue. This feature of hamster fat suggests that it may be a more suitable model system for studies dealing with cellular regulation of lipolysis than is the more commonly used rat fat cell system.

The presence of an inhibitory α-adrenergic influence on cAMP levels and lipolysis in hamster adipose tissue was first documented by Hittelman and his colleagues (10–12), who reported that exposure of hamster adipocytes to the α-adrenergic blocking drug phentolamine caused increased accumulation of cAMP over that measured in the presence of norepinephrine alone. They concluded (10–12) that phentolamine acted by removing an inhibitory influence upon cAMP levels exerted by the α-adrenergic activity of norepinephrine. Subsequently, work from our laboratory (13, 24) demonstrated that exposure of hamster adipocytes to methoxamine and phenylephrine, two relatively specific α-adrenergic agents, decreased lipolysis and under certain conditions partially suppressed cAMP levels. The present data showing that phentolamine increases the activity ratio of protein kinase in concert with increased cAMP levels and conversely that phenylephrine causes a decline in the activity of protein kinase that is associated with a decrease in both cellular cAMP levels and

[1] Schimmel, R. J., and C. A. Buhlinger. Unpublished observations.

in the rate of lipolysis, provides support for a mechanism of action of α-adrenergic stimuli that involves a cAMP-dependent drop in the activity of the protein kinase enzyme. However, evidence has been presented from this laboratory (24) suggesting that the antilipolytic effect of α-adrenergic stimuli cannot be accounted for entirely by a lowering of cellular cAMP levels. It should be noted that lipolysis failed to increase following exposure of fat pads to phentolamine despite large increases in the protein kinase activity ratio in the cAMP level. Virtually identical data were reported by Rosak and Hittelman (11). We attribute the failure of lipolysis to increase in association with increased protein kinase activity and the actual inhibition of lipolysis at high concentrations of phentolamine to an effect of the α-agonist that is unrelated to its actions on the alpha receptor site. Lipolysis activated with ACTH, theophylline, and dibutyryl cAMP as well as norepinephrine is inhibited by phentolamine (8, 11, 25). This observation, coupled with the finding that addition of phentolamine to homogenates of adipose tissue reduces lipase activity, suggests that this drug inhibits the activation of lipase by cAMP (26).

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REFERENCES

1. Steinberg, D. 1976. Interconvertible enzymes in adipose tissue regulated by cyclic AMP-dependent protein kinase. *In* Advances in Cyclic Nucleotide Research. P. Greengard and G. A. Robison, editors. Raven Press, New York. **7:** 157–198.
2. Corbin, J. D., S. L. Kelly, T. R. Soderling, and C. R. Park. Hormonal regulation of adenosine 3′,5′-monophosphate-dependent protein kinase. *In* Advances in Cyclic Nucleotide Research. G. I. Drummond, P. Greengard, and G. A. Robison, editors. Raven Press, New York. **5:** 265–279.
3. Huttunen, J. K., and D. Steinberg. 1971. Activation and phosphorylation of purified adipose tissue hormone sensitive lipase by cyclic AMP-dependent protein kinase. *Biochim. Biophys. Acta.* **239:** 411–427.
4. Ho, R. J., and E. W. Sutherland. 1971. Formation and release of a hormone antagonist by rat adipocytes. *J. Biol. Chem.* **246:** 6822–6827.
5. Manganiello, V. C., F. Murad, and M. Vaughan. 1971. Effects of lipolytic and antilipolytic agents on cyclic 3′,5′-adenosine monophosphate in fat cells. *J. Biol. Chem.* **246:** 2195–2202.
6. Schimmel, R. J. 1974. Responses of adipose tissue to sequential lipolytic stimuli. *Endocrinology.* **94:** 1372–1380.
7. Birnbaum, R. S., and H. M. Goodman. 1977. Studies on the mechanisms of epinephrine stimulation of lipolysis. *Biochim. Biophys. Acta.* **496:** 292–301.
8. Schwabe, U., and R. Ebert. 1972. Different effects of lipolytic hormones and phosphodiesterase inhibitors on cyclic 3′,5′-AMP levels in isolated fat cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **274:** 287–298.
9. Soderling, T. R., J. D. Corbin, and C. R. Park. 1973. Regulation of adenosine 3′,5′-monophosphate-dependent protein kinase II. Hormonal regulation of the adipose tissue enzyme. *J. Biol. Chem.* **248:** 1822–1829.
10. Hittelman, K. J., C. F. Wu, and R. W. Butcher. 1973. Control of cyclic AMP levels in isolated fat cells from hamsters. *Biochim. Biophys. Acta.* **304:** 188–196.
11. Rosak, C., and K. J. Hittelman, 1977. Characterization of lipolytic responses of isolated white adipocytes from hamsters. *Biochim. Biophys. Acta.* **496:** 458–474.
12. Hittelman, K. J., and R. W. Butcher. 1973. Effects of antilipolytic agents and α-adrenergic antagonistics on cyclic AMP metabolism in hamster white adipocytes. *Biochim. Biophys. Acta.* **316:** 403–410.
13. Schimmel, R. J. 1976. Roles of alpha and beta adrenergic receptors in control of glucose oxidation in hamster epididymal adipocytes. *Biochim. Biophys. Acta.* **428:** 379–387.
14. Giudicelli, Y., B. Agli, D. Bralle, and E. Nordman. 1977. Influence of α-adrenergic blocking agents on cyclic AMP, cyclic GMP and lipolysis in hamster white fat cells. *FEBS Lett.* **83:** 225–230.
15. Corbin, J. D., T. R. Soderling, and C. R. Park. 1973. Regulation of adenosine 3′,5′-monophosphate-dependent protein kinase I. Preliminary characterization of the adipose tissue enzyme in crude extracts. *J. Biol. Chem.* **248:** 1813–1821.
16. Corbin, J. D., S. L. Keely, and C. R. Park. 1975. The distribution and dissociation of cyclic adenosine 3′-5′-monophosphate-dependent protein kinases in adipose, cardiac and other tissues. *J. Biol. Chem.* **250:** 218–225.
17. Sherline, P., A. Lynch, and W. H. Glinsmann. 1972. Cyclic AMP and adrenergic receptor control of rat liver glycogen metabolism. *Endocrinology.* **91:** 680–690.
18. Gilman, A. G. 1970. A protein binding assay for adenosine 3′:5′-cyclic monophosphate. *Proc. Natl. Acad. Sci. USA* **68:** 305–312.
19. Mashiter, K., G. D. Mashiter, R. L. Hauger and J. B. Field. 1973. Effects of cholera and *E. coli* enterotoxins on cyclic adenosine 3′,5′-monophosphate levels and intermediary metabolism in the thyroid. *Endocrinology.* **92:** 541–549.
20. Wieland, O. 1957. Eine enzymatische Methode zur Bestiminung von Glycerin. *Biochem. Z.* **329:** 313–319.
21. Correze, C., D. Auclair, and J. Nunez. 1976. Additive effects of norepinephrine and cyclic AMP on the activation of the protein kinase from adipose tissue. *Mol. Cell Endocrinol.* **5:** 339–347.
22. Skala, J. P., and B. L. Knight. 1977. Protein kinase in brown adipose tissue of developing rats. State of activation of protein kinase during development and cold exposure and its relationship to adenosine 3′:5′-monophosphate, lipolysis and heat production. *J. Biol. Chem.* **252:** 1064–1070.

23. Walsh, D. A., C. D. Ashby, C. Gonzalez, D. Calkins, E. H. Fisher, and E. G. Krebs. 1971. Purification and characterization of a protein inhibitor of adenosine 3′,5′-monophosphate-dependent protein kinases. *J. Biol. Chem.* **246:** 1977–1985.
24. Schimmel, R. J. 1979. Inhibition of lipolysis in hamster epididymal adipocytes by selective α-adrenergic agents. Evidence for cyclic AMP-dependent and independent mechanisms. *Biochim. Biophys. Acta.* **587:** 217–226.
25. Westermann, E., and K. Stock. 1970. Inhibitors of lipolysis: potency and mode of action of α- and β-adrenolytics, methoxamine derivatives, prostaglandin E 1 and phenylisopropyl adenosine. *Horm. Metab. Res. Suppl.* **2:** 47–54.
26. Schreibman, P. H., D. E. Wilson, and R. A. Arky, 1971. Effect of antilipolytic compounds on cyclic 3′,5′-adenosine monophosphate activation of adipose tissue lipolysis. *Diabetes.* **20:** 146–150.