ACTIVATION OF HORMONE-SENSITIVE LIPASE FROM HUMAN ADIPOSE TISSUE BY CYCLIC AMP-DEPENDENT PROTEIN KINASE

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SUMMARY

Lipase activation requiring cyclic-3',5'-adenosine monophosphate and ATP was demonstrated in crude fractions of human adipose tissue homogenates. Activation was totally blocked by addition of the specific protein kinase inhibitor. Levels of endogenous protein kinase were adequate to support clear-cut activation but in partially purified preparations addition of exogenous (rabbit muscle) kinase further enhanced activation. When tissue was treated with epinephrine prior to homogenization the degree of activation in partially purified fractions was distinctly reduced. The mechanism of activation of hormone-sensitive lipase in human adipose tissue is thus shown, like that in rat adipose tissue, to be linked to a cyclic AMP-dependent protein kinase.

INTRODUCTION

A good deal of interest attaches to the regulation of fatty acid mobilization in man because of its relevance in diabetic ketoacidosis, some forms of hyperlipoproteinemia and other pathophysiologic states. Studies in vivo and in vitro amply document the responsiveness of human adipose tissue to a variety of hormones, and indirect evidence has implicated cyclic adenosine-3',5'-monophosphate (cAMP) in the process (1,2). Recently, hormone-sensitive lipase from rat adipose tissue has been partially purified and its activation has been shown to be effected via cAMP-dependent protein kinase (3,4). No report has thus far appeared on the operation of a similar mechanism in adipose tissue of other species and our own attempts, using conditions effective in rat preparations, have been equivocal using adipose tissues from rabbit, dog, sheep and pig. We now find that lipase activation dependent on cAMP and protein kinase can be readily demonstrated in crude subcellular fractions of human fat, using conditions similar to those effective in rat preparations.

MATERIALS AND METHODS

Adipose tissue biopsies (omental, pannicular or breast) were obtained at surgery (cholecystectomy, intestinal resection or mastectomy) and studies were begun within a few hours. Slices were incubated for 1 hour at 37° C under 95% O_2 - 5% CO_2 in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 3% bovine serum albumin. The tissue was rinsed in 0.85% NaCl and then fragmented for 30 sec in a Waring blender with 1.5 volumes of ice cold 0.25 M sucrose containing 1 mM EDTA and 10 mM Tris buffer, pH 7.0. The slurry from the Waring blender was homogenized further using a glass-Teflon homogenizer (ten strokes). The final homogenate was centrifuged either at 16,000 x g or 120,000 x g for 30 min. The infranatant fluid layer (below the floating fat cake and above the pellet) was aspirated and carefully adjusted to pH 5.2 by addition of 0.1 N acetic acid while stirring at ice temperature. After 15 min the sample was centrifuged at 900 x g for 15 min. The pellet was taken up in a volume of buffer equal to one-twentieth that of the original infranatant fluid sample. This is designated the pH 5.2 fraction. Lipase specific activity in this fraction was 5 to 10 times that of the original tissue extracts and recoveries ranged from 60 to 100%.

Protein kinase was partially purified from rabbit skeletal muscle by the procedure of Walsh et al. through the first DEAE-cellulose chromatography step (5). Protein kinase inhibitor was also obtained from rabbit skeletal muscle by the method of Walsh et al. (6). Labeled triolein (1-^{14}C-oleic acid in all three positions) was purchased from DHOM Products Ltd., North Hollywood, California.

Hormone-sensitive lipase was assayed using a gum arabic ^{14}C-triolein emulsion as described previously (4). Release of ^{14}C-FFA was linear for at least 1 hour at 30° C. Activation was carried out for 10 min at 30° C in a final volume of 0.2 ml (designated as complete system) containing: ATP, 0.5 mM; magnesium acetate, 5 mM; cAMP, 10 μM; protein kinase, 8.8 μg/ml; Tris buffer, pH 7.4, 10 mM; EGTA, 0.5 mM; dithio-

threitol, 1 mM; theophylline, 1 mM; and 0.1 ml of enzyme. In addition, where indicated, an ATP-regenerating system was included (phosphoenolpyruvate, 2.5 mM; pyruvate kinase, 20 U/ml; and KCl, 50 mM). Activation was stopped by diluting into 2 ml of an assay system containing: 30 mM EDTA; 40 mM sodium phosphate buffer, pH 6.8; 2% bovine serum albumin; and 0.83 mM ^{14}C-triolein.

RESULTS AND DISCUSSION

Preliminary experiments showed that lipase activity against triolein in the 16,000 x g infranatant fluid was optimal at pH 7.0, similar to that in rat adipose tissue (7). Activity at pH 8.4 was only 1/5 that at pH 7.0, indicating that contribution of lipoprotein lipase under the assay conditions used was small. This was expected since no serum was added during assay and since EDTA and phosphate inhibit lipoprotein lipase (8). Total activity in the 16,000 x g fraction was very low, only 1/10 to 1/20 that in similar fractions from rat adipose tissue. All subsequent studies were done with the pH 5.2 isoelectric precipitate as described under Methods.

As shown in Table I, the activity of enzyme prepared from the 16,000 x g infranatant fraction was increased 56% by the complete activation system in the presence of an ATP-regenerating system. In the absence of the ATP-regenerating system an increase of only 37% was obtained, and on omission of ATP activation was marginal.

As shown in Table II, activation in the 16,000 x g infranatant fraction was just as great without addition of exogenous protein kinase as with it. In the pH 5.2 precipitate there was activation without added protein kinase but this was significantly enhanced by adding exogenous kinase. When the pH 5.2 precipitate was prepared from the 120,000 x g fluid fraction activation no longer required the ATP-regenerating system, which was omitted from all subsequent studies. Addition of exogenous kinase to the pH 5.2 fraction gave only a modest increase in activation (Table III). Evidence for the dependency of the activation on protein kinase acitivity was obtained by showing that it was

TABLE I

CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVATION OF LIPASE IN THE pH 5.2 ISOELECTRIC PRECIPITATE FROM A 16,000 x g INFRANATANT FRACTION: REQUIREMENT FOR AN ATP-REGENERATING SYSTEM*

Additions	ATP-regenerating system**	nEqFFA/ mg protein/hour***	Percentage Activation
Complete system**** minus PK and cAMP	+	25	-
Complete system	+	39	56
Complete system minus PK and cAMP	-	27	-
Complete system	-	37	37
Complete system minus ATP, PK and cAMP	+	24	-
Complete system minus ATP	+	28	17

*Omental fat, 19-year old female undergoing cholecystectomy
**Phophoenolpyruvate, 2.5 mM; pyruvate kinase, 20 U/ml; KCl, 50 mM
***^{14}C-FFA expressed in nanoequivalent per milligram protein per hour
****Essentially ATP-Mg^{++}, protein kinase (PK) and cAMP (see methods for details)

completely blocked by adding an excess of protein kinase inhibitor, 8 µg/ml (Table III). The latter was shown not to affect lipase activity directly. The results shown in Table III also demonstrate that activation required both ATP and cAMP. Substitution of GTP, UTP, CTP or ITP for ATP yielded little or no activation; substitution of cyclic GMP, cyclic UMP or cyclic IMP for cyclic AMP was ineffective.

To test whether the activation demonstrated in these subcellular fractions reflected

TABLE II

LIPASE ACTIVATION WITH AND WITHOUT ADDITION OF EXOGENOUS PROTEIN KINASE*

Fraction	Complete system minus protein kinase	Complete system including protein kinase
	Percentage activation ± S.E.**	
16,000 x g infranatant	50 ± 4.2 (n=4)	54 ± 3.9 (n=10)
pH 5.2 fraction from 16,000 x g infranatant	32 ± 6.8 (n=4)	64 ± 8.0 (n=9)

*ATP-regenerating system used throughout
**Percentage activation calculated based on activity in "complete system minus PK and cAMP"

the mechanism of activation of lipase by hormones in intact cells, samples of tissue were incubated prior to homogenization either with or without epinephrine in the medium. As shown in Table IV, the activation obtained in preparations made from epinephrine-treated tissue was much smaller than that in similar preparations from the same tissue not exposed to epinephrine. This strongly supports the interpretation that hormone-stimulated activation proceeds via the cAMP-dependent protein kinase mechanism.

We conclude that the activation of hormone-sensitive lipase in human adipose tissue is mediated by cAMP dependent protein kinase. The activation specifically requires ATP and probably depends on phosphorylation of the lipase as demonstrated for rat adipose tissue lipase (4).

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TABLE III

INHIBITION OF LIPASE ACTIVATION BY ADDITION OF THE SPECIFIC PROTEIN KINASE INHIBITOR (PKI)*

Additions	Lipase activity (nEqFFA/mg protein/hr)	Percentage activation**
None	110	-
Complete system	182	67
Complete system minus cAMP	107	0
Complete system minus PK & cAMP	109	0
Complete system minus ATP	102	0
Complete system plus PKI	106	0
Complete system minus PK & cAMP plus PKI	110	0
Complete system minus PK	166	52
Complete system minus PK plus PKI	108	0

*pH 5.2 precipitate fraction prepared from 120,000 x g infranatant fraction of breast fat homogenate (54 year old female undergoing mastectomy for carcinoma)

**Percentage activation calculated based on activity in "complete system minus PK and cAMP"

TABLE IV

EFFECT OF EPINEPHRINE TREATMENT

ON KINASE-DEPENDENT ACTIVATION OF LIPASE*

Additions	Additions during activation step	Lipase activity (nEqFFA/mg/hr)	Percentage activation
None	Complete system minus PK and cAMP	109	-
	Complete system	182	67
	Complete system minus PK	166	52
Epinephrine $1x10^{-5}M$	Complete system minus PK and cAMP	164	-
	Complete system	195	19
	Complete system minus PK	183	12

*Same tissue source as in Table III. Tissue incubated 1 hour at 37° C prior to homogenization and then pH 5.2 precipitate prepared from 120,000 x g fraction

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REFERENCES

1. Burns, T.W., Langley, P.E. and Robison, A.G., Ann. N.Y. Acad. Sci, 185, 115 (1971).
2. Moskowitz, J. and Fain, J.N., J. Clin. Invest., 8, 1802 (1969).
3. Huttunen, J.K., Steinberg, D. and Mayer, S.E., Proc. Nat. Acad. Sci., Wash., 67, 290 (1970).
4. Huttunen, J.K. and Steinberg, D., Biochim. Biophys. Acta, 239, 411 (1971).
5. Walsh, D.A., Perkins, J.P. and Krebs, E.G., J. Biol. Chem., 243, 3763 (1968).
6. Walsh, D.A., Ashby, C.D., Gonzalez, C., Calkins, D., Fischer, E.H. and Krebs, E.G., J. Biol. Chem., 246, 1977 (1971).
7. Huttunen, J.K., Aquino, A.A. and Steinberg, D., Biochim. Biophys. Acta, 224, 295 (1970).
8. Korn, E.D. and Quigley, T.W., J. Biol. Chem., 226, 833 (1957).