0013-7227/03/$15.00/0
Printed in U.S.A.

Endocrinology 144(6):2404–2408
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doi: 10.1210/en.2002-0007

In Vitro Inhibition of Insulin-Degrading Enzyme by Long-Chain Fatty Acids and Their Coenzyme A Thioesters

FREDERICK G. HAMEL, JENNIFER L. UPWARD, AND ROBERT G. BENNETT

Research Service (F.G.H., J.L.U., R.G.B.), Department of Veterans Affairs Medical Center, Omaha, Nebraska 68105; and Departments of Internal Medicine (F.G.H., R.G.B.) and Pharmacology (F.G.H.), University of Nebraska Medical Center, Omaha, Nebraska 68198

Insulin-degrading enzyme is responsible for initiating insulin degradation in cells, but little is known about the factors controlling its activity. Because obesity and high levels of free fatty acids decrease insulin clearance, we examined the effect of some common free fatty acids and their acyl-coenzyme A thioesters on insulin-degrading enzyme partially purified from the livers of male Sprague Dawley rats. Octanoic acid (C8:0) had no effect on activity. Long-chain free fatty acids (C16–C20) inhibited between 50% and 90% of the insulin degradation with IC_{50} values in the range of 10–50 μM. In general, the corresponding acyl-coenzyme A thioesters had lower IC_{50} values and were slightly more efficacious. ^{125}I-insulin cross-linking studies showed free fatty acids did not inhibit hormone binding to insulin-degrading enzyme. Kinetic analysis showed a noncompetitive type of inhibition. Furthermore, fatty acids eliminated the ability of insulin to inhibit the proteasome. These results suggest that when intracellular long-chain fatty acid concentrations are elevated, they may act directly on insulin-degrading enzyme to decrease insulin metabolism and alter insulin action in intact cells. This mechanism may contribute to the hyperinsulinemia and insulin resistance seen with elevated fatty acids and obesity. (*Endocrinology* 144: 2404–2408, 2003)

OBESITY AND ELEVATED levels of free fatty acids (FFAs) are associated with insulin resistance and the development of type 2 diabetes mellitus. One consequence of these conditions is a decrease in insulin processing. Hepatic insulin clearance is reduced in overfed rats and negatively correlated with hepatic triglyceride content (1). Similar results were found with bovine cells (2). Severely obese humans also demonstrate decreased insulin clearance, which is significantly improved with weight loss (3). It is not just high tissue lipid content that is responsible for this effect; addition of FFAs to rat liver perfusate acutely decreased hepatic insulin removal by up to 40% (4). *In vivo* experiments in dogs have also shown that elevated circulating FFAs decrease peripheral insulin clearance and hepatic insulin extraction (5). *In vitro* experiments with FFAs and isolated hepatocytes have shown a decrease in insulin binding and degradation (6–8). These studies showed no effect of FFAs on partially purified insulin receptors and support the idea that receptor turnover and insulin processing were affected. Because insulin-degrading enzyme is the enzyme primarily responsible for initiating insulin degradation in endosomes (9), this is an obvious possible target for the action of FFAs. Furthermore, using the ExPASy web site (http://expasy.cbr.nrc.ca), proteomic tools for pattern and profile searches, the insulin-degrading enzyme is found to possibly possess a "cytosolic fatty-acid binding proteins signature" (Prosite access PS00214). The consensus sequence and corresponding amino acids of the rat and human insulin-degrading enzyme are shown in Fig. 1. The human and rat enzymes are 66% and 77% similar to the consensus sequence. If the intervening space-holding residues are included, the similarity rises to 83% and 89%, respectively. This is a strong indication that insulin-degrading enzyme is capable of binding fatty acids.

Insulin-degrading enzyme is not only important in insulin metabolism but also has effects on control of protein degradation. We previously found that partial purification of insulin-degrading enzyme from cytosol resulted in coisolation of the proteasome, indicating a complex between proteasome and insulin-degrading enzyme (10). We have further shown that insulin acts on this complex to inhibit two of the proteasome's catalytic activities, the chymotrypsin- and trypsin-like activities (11) with a noncompetitive type of inhibition (12). Using a cell-permeable substrate of the proteasome, we have shown that insulin, added to HepG2 hepatoma cells, will inhibit the chymotrypsin-like activity (13, 14). The action of insulin-degrading enzyme on proteasome function applies to both the 20S and 26S forms (15). Thus, insulin-degrading enzyme is important in insulin action as well as hormone degradation. However, little has been published on metabolic control of insulin-degrading enzyme, with one report showing ATP could inhibit the enzyme (16). The effect of elevated fatty acids to inhibit insulin clearance prompted us to determine whether fatty acids had any effect on insulin-degrading enzyme activity. In this study we show that at physiological concentrations, FFAs, and acyl-coenzyme A (CoA) thioesters inhibit insulin-degrading enzyme degradation of insulin and alter its ability to affect proteasome activity.

Abbreviations: CoA, Coenzyme A; FFA, free fatty acid; LLVY, succinyl-leu-leu-val-tyr-7-amido-4-methyl coumarin; LSTR, boc-leu-ser-thr-arg-7-amido-4-methyl coumarin; TCA, trichloroacetic acid.

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                                                      L
G                               L                     I
S          L                    I                     V    cytosolic fatty acid
A          I                    V  L                  M    binding proteins signature
I    F     V          N         M  I                  A    Prosite access # PS00214
V    Y     M          H  F  D   F  V                  K
K  x W  x  F  x x x x G  Y  E x Y  M  x  x            R

K  F  E  F  p  K  A  C  L  N  F  E  F  F  s  P  F  A    aa 566 to 583 of rat IDE

K  K  k  K  p  K  A  C  L  N  F  E  F  F  s  P  F  A    aa 565 to 582 of human IDE
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FIG. 1. Cytosolic fatty acid-binding proteins signature. The figure shows the 18 amino acid fatty acid-binding protein consensus sequence and the matching sequences in the rat and human insulin-degrading enzymes. In the upper portion, the specific required residues are shown in *capital letters* and undefined residues shown as *x*. In the lower portion, *capital letters* indicate the amino acids consistent with the consensus sequence, and *underlined, bold letters* indicate amino acids that match the specific required residues.

Materials and Methods

Materials

Crystalline human insulin and ^{125}I-insulin were provided by Lilly Research Laboratories (Indianapolis, IN). Enzyme-grade ammonium sulfate was purchased from ICN Biomedicals, Inc. (Irvine, CA). The fluorogenic peptides succinyl-leu-leu-val-tyr-7-amido-4-methyl coumarin (LLVY) and boc-leu-ser-thr-arg-7-amido-4-methyl coumarin (LSTR), fatty acids, and acyl-CoA thioesters were obtained from Sigma (St. Louis, MO). All other chemicals were of at least reagent grade.

Enzyme preparation

Male Sprague Dawley rats were maintained and used in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, under a protocol approved by the Subcommittee of Animal Studies and the Research and Development Committee of the Omaha Veterans Affairs Medical Center. Insulin-degrading enzyme/proteasome was prepared from rat liver by ultracentrifugation, ammonium sulfate precipitation, and diethylaminoethyl-Sephacel batch elution similar to that described previously (17).

Assay of insulin degradation

The degradation of ^{125}I-insulin was measured by the trichloroacetic acid (TCA) solubility assay (17). Fatty acids were dissolved in ethanol and added at the indicated concentrations. The final concentration of ethanol was 1% (vol/vol), which altered insulin-degrading enzyme activity by less than 10%. Briefly, an enzyme aliquot was incubated with ^{125}I-[A14]-iodoinsulin in 1 ml Tris (pH 7.4) for 15 min at 37 C. The reaction was stopped by addition of 0.5% (final) BSA and 10% (final) TCA. The samples were centrifuged and the supernatant and pellet counted on a γ counter. Qualitative analysis of the products formed was done by HPLC as described previously (18).

Covalent cross-linking of ^{125}I-insulin to insulin-degrading enzyme

Preparations of partially purified insulin-degrading enzyme were covalently cross-linked to ^{125}I-insulin using the homobifunctional amine-reactive cross-linker disuccininmidyl suberate (Pierce Chemical Co., Rockford, IL) by a procedure described previously (19).

Assay of proteasome activity

The proteasome has a number of peptidolytic activities, which can be assayed by the cleavage of fluorogenic peptides. The degradation of LLVY and LSTR were used as measures of the chymotrypsin-like and trypsin-like activities, respectively, as described previously (11). The enzyme sample was incubated with 13 μM LLVY or LSTR in 1 ml 100 mM Tris (pH 7.5), with additions as noted, for 1 h. The reaction was stopped with 0.2 ml ice cold ethanol and activity expressed as the change in fluorescence over time (excitation and emission wavelengths of 390 nm and 440 nm, respectively).

Data analysis

All data are shown as mean ± SEM. The IC_{50}s and maximal effect were determined by fitting the data to a one-site competition model with nonlinear regression using GraphPad Prism (version 3.02 for Windows, GraphPad Software, Inc., San Diego, CA). Means were compared by ANOVA with Dunnett's multiple comparison posttest. The Dixon plot data were analyzed by fitting the data with linear regression.

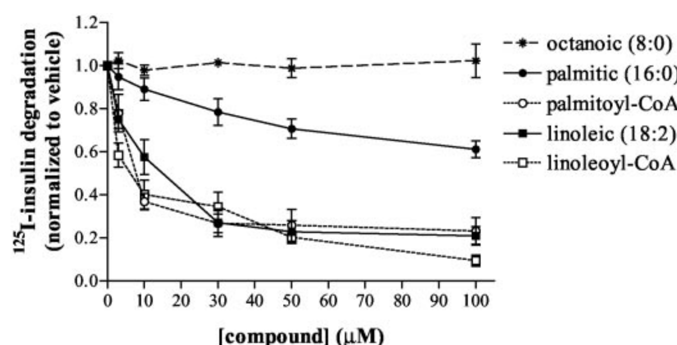

FIG. 2. Inhibition of insulin-degrading enzyme by fatty acid and fatty acyl CoA thioesters. The effect of octanoic acid (n = 4), palmititic acid, (n = 4), palmitoyl-CoA (n = 4), linoleic acid, (n = 5), and linoleoyl-CoA (n = 4) on insulin-degrading activity are shown as indicated. The data are expressed as a ratio to degradation with vehicle (ethanol) alone.

Results

Figure 2 shows the inhibition of insulin-degrading enzyme by selected fatty acids and fatty acyl CoA thioesters. Table 1 shows IC_{50} values and the maximal amount of inhibition by all the compounds tested. Octanoic acid, acetyl-CoA, malonyl-CoA, and lactic acid had no effect. Palmitic acid (16:0), palmitoleic acid (16:1), arachidonic acid (20:4), arachidonoyl-CoA, and docosahexaenoic acid (22:6) had substantial effects but only at the highest concentrations tested. The C18 unsaturated FFAs were the most potent of those we tested. Although the addition of the CoA group to arachidonic acid did not increase its effectiveness, linoleoyl-CoA, oleoyl-CoA, and especially palmitoyl-CoA were more effective than their respective FFAs.

We also tested, by HPLC analysis, the qualitative effect of FFAs on the generation of insulin products. Figure 3 shows representative elution profiles of A14-iodoinsulin fragments after incubation with insulin-degrading enzyme in the absence and presence of linoleic acid. The product patterns were very similar, with no major products missing or additional peaks arising from the inclusion of linoleic acid. The first three peaks were reduced with the FFA-treated enzyme, which would account for the small difference in TCA solubility between the two samples (4.6% *vs.* 3.6%, control *vs.* linoleic acid). These preliminary results suggest that FFAs do not alter the enzyme's preference for the various cleavage sites but generally decrease degradation.

To examine the nature of the inhibition, we cross-linked

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TABLE 1. IC_{50} and maximal inhibition of tested compounds

Compound	IC_{50} (μM)	% Maximal inhibition
Lactic acid		0
Malonyl-CoA		0
Acetyl-CoA		0
Octanoic acid (8:0)		0
Palmitic acid (16:0)	44	55
Palmitoyl-CoA	5	84
Palmitoleic acid (16:1)	30	64
Stearic acid (18:0)		11
Oleic acid (18:1)	31	72
Oleoyl-CoA	6	83
Linoleic acid (18:2)	9	89
Linoleoyl-CoA	4	84
Conjugated linoleic acid (*cis/trans*, 9,11 and 10,12)	7	98
Linolenic acid (18:3)	14	70
Arachidonic acid (20:4)	34	75
Arachidonoyl-CoA	77	60
Docosahexaenoic acid (22:6)	34	61
Nervonic acid (24:1)		14

All compounds were tested on four independent enzyme preparations except: stearic acid, oleic acid conjugated linoleic acid, and docosahexaenoic acid, n = 3; linoleic acid, n = 5; and arachidonic acid, n = 6.

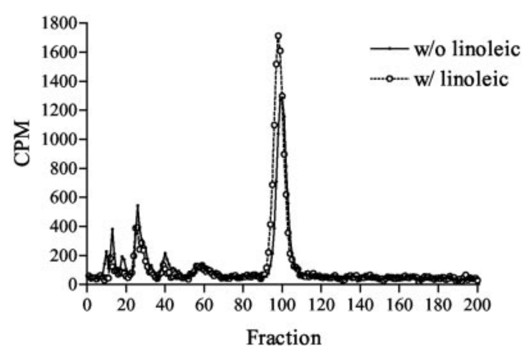

FIG. 3. HPLC analysis of insulin degradation products in the presence and absence of linoleic acid. The figure shows a representative chromatograph of ^{125}I-insulin incubated with partially purified insulin-degrading enzyme for 15 min in the presence or absence of 100 μM linoleic acid. The experiment was performed at least nine times, with TCA solubility varying from 3% to 30% and using three independent enzyme preparations. Similar results have been obtained with palmitoyl-CoA.

insulin to insulin-degrading enzyme in the absence and presence of linoleic acid and linoleoyl-CoA. Figure 4 shows a sample autoradiograph of the 110-kDa band (the molecular weight of insulin-degrading enzyme) and quantification by densitometry. Interestingly, neither compound significantly suppressed cross-linking, and in fact there appears to be a small increase. Similar results were seen with other FFAs (data not shown). This indicates that FFAs inhibit degradation without disrupting binding and suggests a noncompetitive inhibition. Analysis of FFA inhibition by Dixon plot confirms this conclusion (Fig. 5). The inhibitory constants for linoleic acid and palmitoyl-CoA were 23 ± 3 and 15 ± 1 μM, respectively.

We have previously shown that insulin will decrease the chymotrypsin- and trypsin-like activities of the proteasome when it is isolated in a complex with insulin-degrading enzyme. We tested whether fatty acids would have an effect on this inhibition. Figure 6 shows that increasing concentrations of fatty acid inhibit the chymotrypsin-like and trypsin-like activities of the proteasome and insulin will not further decrease those activities. Thus, in the presence of fatty acids, the ability of insulin to inhibit the proteasome is lost.

Discussion

Our data show that FFAs and related acyl-CoA thioesters can inhibit the actions of insulin-degrading enzyme. This inhibition has implications for two aspects of obesity and diabetes, hyperinsulinemia and insulin resistance. Elevated lipids and FFAs have been shown to affect insulin processing at the cellular level (6–8) as well as insulin clearance in whole animals (1–5). Our studies show FFAs noncompetitively inhibit insulin-degrading enzyme without changing the products produced. In the Zucker fatty rat model, palmitoyl-CoA levels are doubled in livers and muscles (20) to a level that would correlate to about a 50% increase in inhibition of insulin-degrading enzyme, based on our data. Because insulin-degrading enzyme is responsible for initiating insulin degradation in endosomes (9), impairment of its action could be responsible for decreased clearance and thereby contribute to the hyperinsulinemia found in insulin-resistant and diabetic conditions in animals and humans.

The inhibition of insulin-degrading enzyme by FFAs may also represent a physiological control mechanism. The FFA concentrations that show significant, though not complete, inhibition are in the low micromolar range. The IC_{50}s of the most potent compounds are around 10 μM, a concentration that is within physiological range and shows similar inhibition of hexokinase (21). Postprandial absorption of FFAs could easily achieve these levels in the liver, decreasing liver clearance and adding to the rise of peripheral insulin concentrations. Thus, FFAs are a likely metabolic control for the action of insulin-degrading enzyme.

However, insulin-degrading enzyme has functions beyond simply degrading hormones, and interruption of its actions could have implications in hormone action and signal transduction and thus insulin resistance. Internalization and processing of insulin are necessary for insulin effects on cellular protein degradation and amino acid transport but not insulin-stimulated glycogen synthesis (22–24). Intracellular insulin, internalized by linking to ricin molecules or microinjection, has biological effects (25–28). It has also been shown that insulin can be internalized and transported to the nucleus, and insulin-degrading enzyme is one of the proteins involved (29–32). The intracellular receptors for androgens and glucocorticoids form a complex with insulin-degrading enzyme that increases their activities (33, 34). Addition of insulin to the insulin-degrading enzyme-steroid receptor complex decreases the receptor's binding of DNA, which agrees with the physiological effect of insulin. We have similarly shown insulin-degrading enzyme forms a complex with the proteasome that increases peptidolytic activity (10, 11, 15, 35). Insulin acts on this complex to cause the dissociation of insulin-degrading enzyme, noncompetitively decreasing two of the proteasome's catalytic activities. These data all support interactions among insulin, insulin-degrading enzyme, and various cytosolic proteins and raise the possibility of an intracellular site of action for insulin (36, 37).

We have shown here that at least one of these interactions,

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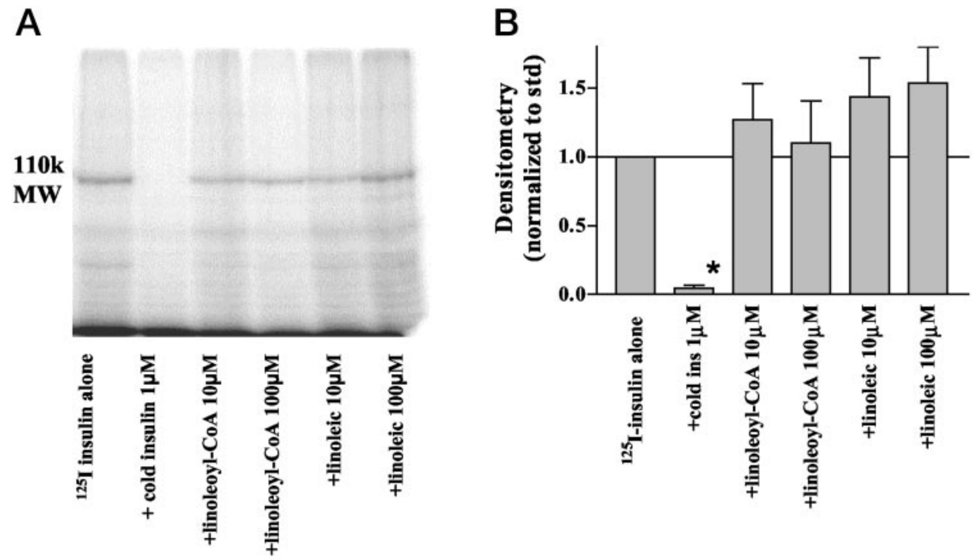

FIG. 4. Effect of linoleic acid and linoleoyl-CoA on ^{125}I-insulin cross-linking to insulin-degrading enzyme. A, A representative autoradiograph showing the effect of 10 μM or 100 μM linoleic acid and linoleoyl-CoA on the cross-linking of ^{125}I-insulin to insulin-degrading enzyme. Inhibition of cross-linking by 1 μM unlabeled insulin is shown as a control. B, The densitometric analysis of six independent experiments. *, $P < 0.05$, compared with ^{125}I-insulin alone.

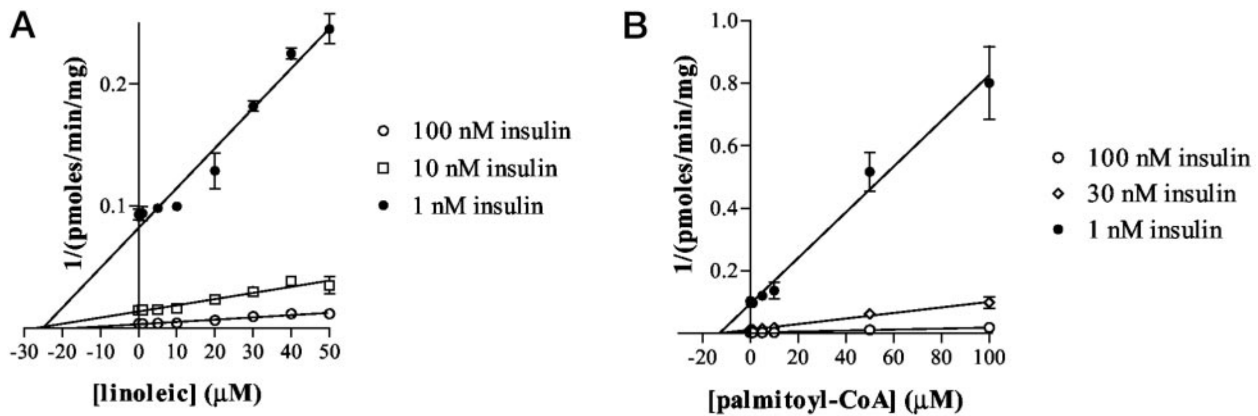

FIG. 5. Dixon plots of linoleic acid and palmitoyl-CoA inhibition of insulin-degrading enzyme. The data are plotted as the inverse of the velocity *vs.* the concentration of inhibitor (linoleic acid, A, or palmitoyl-CoA, B) and represent the means of three experiments. The *lines* show the results of linear regression of the data. The inhibitory constants were determined by the x-intercept.

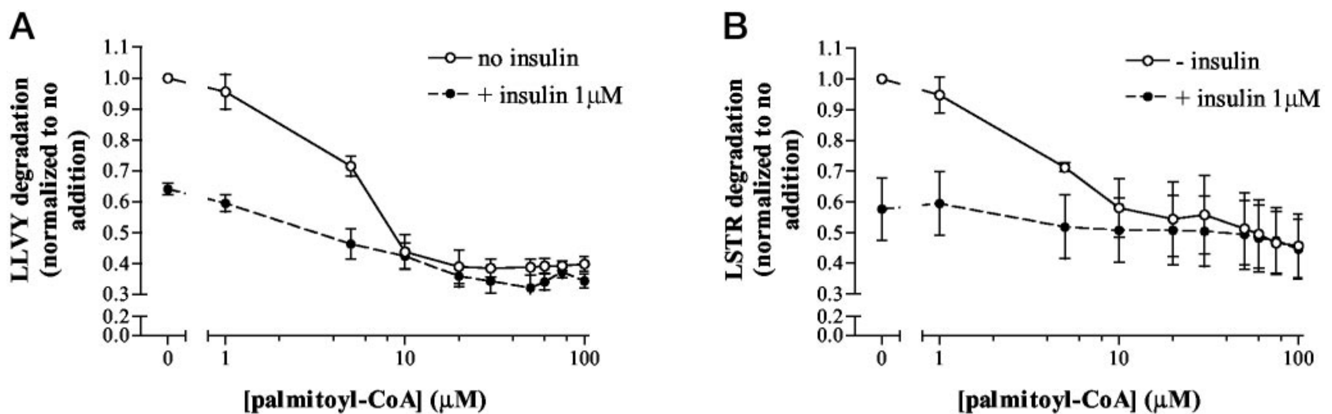

FIG. 6. Loss of insulin-responsive proteasome activity by palmitoyl-CoA. The graph shows the effect of increasing concentrations of palmitoyl-CoA on the chymotrypsin-like (A, n = 4) and trypsin-like (B, n = 4) activities of the proteasome. *Open* and *closed circles* show the effect in the absence and presence of 1 μM insulin, respectively. High concentrations of palmitoyl-CoA do not inhibit the proteasome more than insulin alone. The data are expressed as a ratio to degradation with vehicle alone.

with the proteasome, is affected by FFAs. In the absence of insulin, increasing concentrations of palmitoyl-CoA decrease the activity of the proteasome down to the level of that found with maximal inhibition by insulin (1 μM). We believe that binding of the fatty acid, like that of insulin, causes a dissociation of insulin-degrading enzyme from the proteasome, de-

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creasing peptidolytic activity. Insulin and FFAs are not additive, suggesting a similar mechanism. In metabolic states in which FFAs are increased, protein degradation would be decreased, and, more importantly, the ability of insulin to alter protein degradation via the proteasome would be abrogated. Because insulin's major effect on protein metabolism is to decrease protein degradation, this would be a form of insulin resistance. Thus, FFA inhibition of insulin-degrading enzyme may contribute to insulin resistance by disrupting control of protein metabolism.

Although speculative, the possible implications of our results go beyond insulin and diabetes. Insulin-degrading enzyme has been shown to degrade a number of hormones such as atrial natriuretic peptide, transforming growth factor-α, and amylin (38–40). It has been implicated in the degradation of amyloid β (41) as well as the β-amyloid precursor protein intracellular domain (42). Insulin-degrading enzyme has also been shown to bind to FAK-related nonkinase (43). Thus, insulin-degrading enzyme has a number of potential ligands/substrates with which it interacts. The physiological relevance of these interactions and whether they are altered by FFAs remain to be elucidated.

Acknowledgments

Received November 4, 2002. Accepted February 20, 2003.

Address all correspondence and requests for reprints to: Frederick G. Hamel, Ph.D., Department of Veterans Affairs Medical Center, 4101 Woolworth Avenue, Omaha, Nebraska 68105. E-mail: fghamel@unmc.edu.

This work was supported by funds from the Department of Veterans Affairs Merit Review Program (F.G.H.) and the Bly Memorial Research Fund.

References

1. **Stromblad G, Bjorntorp P** 1986 Reduced hepatic insulin clearance in rats with dietary-induced obesity. Metabolism 35:323–327
2. **Strang BD, Bertics SJ, Grummer RR, Armentano LE** 1998 Relationship of triglyceride accumulation to insulin clearance and hormonal responsiveness in bovine hepatocytes. J Dairy Sci 81:740–747
3. **Jimenez J, Zuniga-Guajardo S, Zinman B, Angel A** 1987 Effects of weight loss in massive obesity on insulin and C-peptide dynamics: sequential changes in insulin production, clearance, and sensitivity. J Clin Endocrinol Metab 64:661–668
4. **Svedberg J, Stromblad G, Wirth A, Smith U, Bjorntorp P** 1991 Fatty acids in the portal vein of the rat regulate hepatic insulin clearance. J Clin Invest 88:2054–2058
5. **Wiesenthal SR, Sandhu H, McCall RH, Tchipashvili V, Yoshii H, Polonsky K, Shi ZQ, Lewis GF, Mari A, Giacca A** 1999 Free fatty acids impair hepatic insulin extraction *in vivo.* Diabetes 48:766–774
6. **Hennes MM, Shrago E, Kissebah AH** 1990 Receptor and postreceptor effects of free fatty acids (FFA) on hepatocyte insulin dynamics. Int J Obes 14:831–841
7. **Svedberg J, Bjorntorp P, Smith U, Lonnroth P** 1990 Free-fatty acid inhibition of insulin binding, degradation, and action in isolated rat hepatocytes. Diabetes 39:570–574
8. **Svedberg J, Bjorntorp P, Smith U, Lonnroth P** 1992 Effect of free fatty acids on insulin receptor binding and tyrosine kinase activity in hepatocytes isolated from lean and obese rats. Diabetes 41:294–298
9. **Hamel FG, Posner BI, Bergeron JJ, Frank BH, Duckworth WC** 1988 Isolation of insulin degradation products from endosomes derived from intact rat liver. J Biol Chem 263:6703–6708
10. **Bennett RG, Hamel FG, Duckworth WC** 1994 Identification and isolation of a cytosolic proteolytic complex containing insulin degrading enzyme and the multicatalytic proteinase. Biochem Biophys Res Commun 202:1047–1053
11. **Duckworth WC, Bennett RG, Hamel FG** 1994 A direct inhibitory effect of insulin on a cytosolic proteolytic complex containing insulin-degrading enzyme and multicatalytic proteinase. J Biol Chem 269:24575–24580
12. **Bennett RG, Hamel FG, Duckworth WC** 1997 Characterization of the insulin inhibition of the peptidolytic activities of the insulin-degrading enzyme-proteasome complex. Diabetes 46:197–203
13. **Hamel FG, Bennett RG, Harmon KS, Duckworth WC** 1997 Insulin inhibition of proteasome activity in intact cells. Biochem Biophys Res Commun 234: 671–674
14. **Duckworth WC, Bennett RG, Hamel FG** 1998 Insulin acts intracellularly on proteasomes through insulin-degrading enzyme. Biochem Biophys Res Commun 244:390–394
15. **Bennett RG, Hamel FG, Duckworth WC** 2000 Insulin inhibits the ubiquitin-dependent degrading activity of the 26S proteasome. Endocrinology 141:2508–2517
16. **Camberos MC, Perez AA, Udrisar DP, Wanderley MI, Cresto JC** 2001 ATP inhibits insulin-degrading enzyme activity. Exp Biol Med (Maywood) 226: 334–341
17. **Duckworth WC, Heinemann M, Kitabchi AE** 1972 Purification of insulin specific protease by affinity chromatography. Proc Natl Acad Sci USA 69: 3698–3702
18. **Hamel FG, Peavy DE, Ryan MP, Duckworth WC** 1987 HPLC analysis of insulin degradation products from isolated hepatocytes. Effects of inhibitors suggest intracellular and extracellular pathways. Diabetes 36:702–708
19. **Shii K, Baba S, Yokono K, Roth RA** 1985 Covalent linkage of 125I insulin to a cytosolic insulin degrading enzyme. J Biol Chem 260:6503–6506
20. **Murakami K, Ide T, Nakazawa T, Okazaki T, Mochizuki T, Kadowaki T** 2001 Fatty-acyl-CoA thioesters inhibit recruitment of steroid receptor co-activator 1 to α and γ isoforms of peroxisome-proliferator-activated receptors by competing with agonists. Biochem J 353:231–238
21. **Thompson AL, Cooney GJ** 2000 Acyl-CoA inhibition of hexokinase in rat and human skeletal muscle is a potential mechanism of lipid-induced insulin resistance. Diabetes 49:1761–1765
22. **Draznin B, Trowbridge M** 1982 Inhibition of intracellular proteolysis by insulin in isolated rat hepatocytes. Possible role of internalized hormone. J Biol Chem 257:11988–11993
23. **Peavy DE, Edmondson JW, Duckworth WC** 1984 Selective effects of inhibitors of hormone processing on insulin action in isolated hepatocytes. Endocrinology 114:753–760
24. **Trowbridge M, Draznin B** 1986 Insulin internalization and intracellular protein degradation: a quantitative correlation. Horm Metab Res 18:156–158
25. **Hofmann CA, Lotan RM, Ku WW, Oeltmann TN** 1983 Insulin-ricin B hybrid molecules mediate an insulin-associated effect on cells which do not bind insulin. J Biol Chem 258:11774–11779
26. **Miller DS** 1988 Stimulation of RNA and protein synthesis by intracellular insulin. Science 240:506–509
27. **Miller DS** 1989 Stimulation of protein synthesis in stage IV *Xenopus* oocytes by microinjected insulin. J Biol Chem 264:10438–10446
28. **Miller DS, Sykes DB** 1991 Stimulation of protein synthesis by internalized insulin. J Cell Physiol 147:487–494
29. **Harada S, Smith RM, Smith JA, Jarett L** 1993 Inhibition of insulin-degrading enzyme increases translocation of insulin to the nucleus in H35 rat hepatoma cells: evidence of a cytosolic pathway. Endocrinology 132:2293–2298
30. **Harada S, Smith RM, Jarett L** 1994 1, 10-Phenanthroline increases nuclear accumulation of insulin in response to inhibiting insulin degradation but has a biphasic effect on insulin's ability to increase mRNA levels. DNA Cell Biol 13:487–493
31. **Shah N, Zhang S, Harada S, Smith RM, Jarett L** 1995 Electron microscopic visualization of insulin translocation into the cytoplasm and nuclei of intact H35 hepatoma cells using covalently linked Nanogold-insulin. Endocrinology 136:2825–2835
32. **Harada S, Smith RM, Jarett L** 1999 Mechanisms of nuclear translocation of insulin. Cell Biochem Biophys 31:307–319
33. **Kupfer SR, Marschke KB, Wilson EM, French FS** 1993 Receptor accessory factor enhances specific DNA binding of androgen and glucocorticoid receptors. J Biol Chem 268:17519–17527
34. **Kupfer SR, Wilson EM, French FS** 1994 Androgen and glucocorticoid receptors interact with insulin degrading enzyme. J Biol Chem 269:20622–20628
35. **Hamel FG, Bennett RG, Duckworth WC** 1998 Regulation of multicatalytic enzyme activity by insulin and the insulin-degrading enzyme. Endocrinology 139:4061–4066
36. **Duckworth WC, Bennett RG, Hamel FG** 1997 The significance of intracellular insulin to insulin action. J Investig Med 45:20–27
37. **Duckworth WC, Bennett RG, Hamel FG** 1998 Insulin degradation: progress and potential. Endocr Rev 19:608–624
38. **Muller D, Baumeister H, Buck F, Richter D** 1991 Atrial natriuretic peptide (ANP) is a high-affinity substrate for rat insulin-degrading enzyme. Eur J Biochem 202:285–292
39. **Hamel FG, Gehm BD, Rosner MR, Duckworth WC** 1997 Identification of the cleavage sites of transforming growth factor α by insulin-degrading enzymes. Biochim Biophys Acta 1338:207–214
40. **Bennett RG, Duckworth WC, Hamel FG** 2000 Degradation of amylin by insulin-degrading enzyme. J Biol Chem 275:36621–36625
41. **Mukherjee A, Song E, Kihiko-Ehmann M, Goodman Jr JP, Pyrek JS, Estus S, Hersh LB** 2000 Insulysin hydrolyzes amyloid β peptides to products that are neither neurotoxic nor deposit on amyloid plaques. J Neurosci 20:8745–8749
42. **Edbauer D, Willem M, Lammich S, Steiner H, Haass C** 2002 Insulin-degrading enzyme rapidly removes the β-amyloid precursor protein intracellular domain (AICD). J Biol Chem 277:13389–13393
43. **Liu Y, Loijens JC, Martin KH, Karginov AV, Parsons JT** 2002 The association of ASAP1, an ADP ribosylation factor-GTPase activating protein, with focal adhesion kinase contributes to the process of focal adhesion assembly. Mol Biol Cell 13:2147–2156

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