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Preliminary report: inhibition of cellular proteasome activity by free fatty acids

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Abstract

There is evidence in animal studies that free fatty acids (FFA) can decrease protein degradation, but the exact mechanism is not known. We have shown that FFA can inhibit proteasome activity in vitro by interacting with insulin-degrading enzyme. Here we show that FFA can also inhibit the proteasome in whole cells. HepG2 cells were treated with various FFA, and proteasome activity was measured using a cell-permeable substrate for the chymotrypsin-like activity. Octanoic acid, a medium-chain fatty acid, did not affect proteasome activity. However, oleic and linoleic acids inhibited the chymotrypsin-like activity up to 80%, with approximate IC50s of 80 and 40 μmol/L, respectively. Insulin also inhibited but was not additive with the FFA, suggesting that they work through the same mechanism. These results show that the proteasome can be inhibited by FFA in whole cells and suggest that insulin-degrading enzyme may mediate this effect. This mechanism may be applicable to whole animals and represents a means to integrate hormonal and nutrient signals on the control of protein degradation.
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The body can obtain energy primarily from 3 sources: carbohydrates, fatty acids, and amino acids/proteins. Efficient cellular metabolism thus requires balancing the use of these 3 sources depending on their availability. The hypothesis of Randle et al [1] provides an explanation for the balance between glucose and fatty acid use depending on the relative supply of fatty acids. Likewise, amino acids, supplied by the degradation of endogenous proteins, can be converted into glucose when carbohydrate stores are low. Thus, a system exists to balance protein and carbohydrate metabolism. However, a mechanism for balancing protein and lipid (free fatty acids [FFA]) metabolism has not been clearly defined.

Although there is conflicting evidence, some studies suggest that such a balance does exist to spare protein degradation when sufficient FFA are available. Muscle from rats fed a diet high in ω-3 fatty acids showed a decrease in net protein degradation, which was not significantly further lowered by the addition of insulin [2]. Similarly, fasted dogs infused with triglycerides showed an increase in FFA levels and a decrease in leucine carbon flux and leucine oxidation [3]. Conversely, nicotinic acid infusion lowered FFA and increased leucine carbon flux and oxidation. Fery and coworkers [4,5] showed that acipimox, which inhibits lipolysis and decreases FFA availability, significantly increased protein catabolism. Similarly, Norrelund et al [6] used acipimox to demonstrate that growth hormone decreases protein breakdown during fasting by stimulating lipolysis and mobilizing FFA. A study of parenteral feeding compared glucose alone vs isoenergetic glucose plus lipid [7]. Protein breakdown and turnover were significantly higher in the glucose-alone group, indicating that the lipids in the diet decreased protein metabolism. Taken together, these studies indicate that there is an inverse relationship between FFA levels and protein breakdown.

One of the primary mechanisms for protein degradation in cells is the proteasome [8-10]. It is responsible for degrading oxidized and misfolded proteins, as well as controlling the levels of key regulatory proteins. We have demonstrated that insulin-degrading enzyme (IDE) can associate with the proteasome [11-15]. Addition of insulin causes a dissociation

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of IDE from the proteasome and a decrease in the chymotrypsin-like and trypsin-like activities. We have also shown that FFA can inhibit IDE in vitro and that this interaction results in decreased proteasome activity [16]. Here we present evidence that this inhibitory effect of FFA is also seen in whole cells.

1. Materials and methods

HepG2 cells were purchased from ATCC (Rockville, MD). Dulbecco modified Eagle medium was purchased from Sigma (St Louis, MO). Fetal bovine serum was obtained from Intergen (Purchase, NY). Methoxysuccinyl-phe-leu-phe-7-amido-4-trifluoromethyl coumarin (FLF) was purchased from MP Biomedicals (Irvine, CA). The proteasome inhibitor *N*-boc-ile-glu(*O*-*t*-butyl)-ala-leucinal was from Peptide International (Louisville, KY). The proteasome inhibitor lactacystin was from EJ Corey (Harvard University, Cambridge, MA). All other reagents were of at least reagent grade.

1.1. Cellular proteasome activity

After fatty acid or insulin treatment (30 minutes), peptide degradation was assessed with the membrane-permeable substrate FLF, as described previously [14,15]. The measurements were done with triplicate wells, and the n of the experiments represents separate plates run on different days. Values shown are means ± SEM. Statistical analysis was done using analysis of variance with either a Dunnett or Tukey posttest, as appropriate, using GraphPad Prism version 5.0 (GraphPad, San Diego, CA).

2. Results

Fig. 1A shows the dose-dependent inhibition of FLF degradation by FFA. Octanoic acid had essentially no effect, but linoleic acid (18:2) and oleic acid (18:1) showed significant inhibition at or greater than 40 and 80 μmol/L, respectively. Arachidonic acid (20:4) and nervonic acid (24:1) both inhibited less than 5% at 100 μmol/L (data not shown). Fig. 1B shows the inhibition of FLF degradation by insulin, palmitate, and the two combined. As can be seen, insulin and palmitate were equivalently effective; and the combination showed no further inhibition. The effect of insulin was similarly not additive to that of linoleic acid (data not shown). Thus, FFA and insulin can inhibit proteasome activity in the whole cell, probably through the same mechanism.

3. Discussion

To take the maximum advantage of the available nutrients as energy sources, cells must balance their use of carbohydrates, fatty acids, and amino acids. Mechanisms

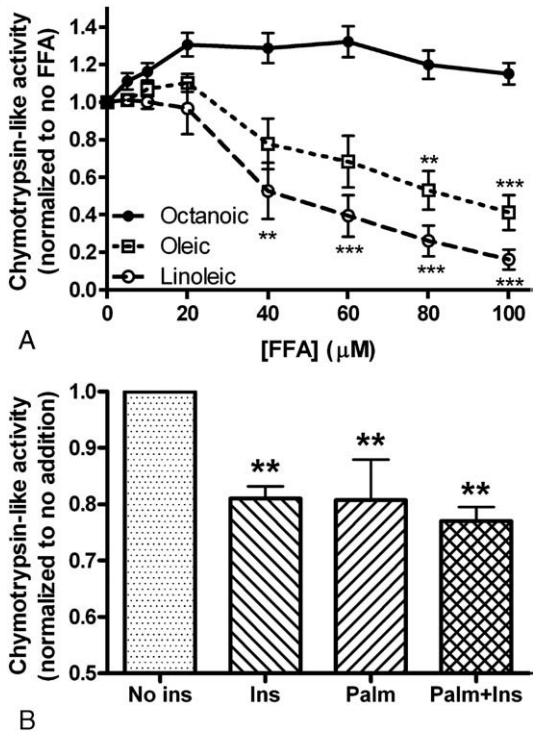

Fig. 1. Inhibition of cellular FLF degrading activity by FFA and insulin. A, Long-chain FFA significantly inhibit the chymotrypsin-like activity in HepG2 cells in a dose-dependent manner. The medium-length octanoic acid had no inhibitory effect (n = 6). B, Insulin (10^{-7} mol/L) and palmitic acid (100 μmol/L) inhibit the proteasome, but are not additive in effect (n = 7 for no insulin and insulin, n = 5 for palmitic acid and palmitic acid + insulin). **P less than .01; ***P less than .001.

have been proposed for balancing carbohydrates with fatty acids; and in times of shortage, glucose can be made from amino acids. However, if fatty acids are present, there is less need to convert amino acids to carbohydrates. A reciprocal mechanism between fatty acid availability and amino acid metabolism is lacking. Amino acid availability is dependent, at least in part, on protein degradation. Thus, control of protein degradation would be a means of regulating amino acid availability and thereby its use in providing an energy source. There is evidence that elevated FFA levels in animals decrease protein turnover, but the exact mechanism has not been elucidated. Our previous study and the findings presented here indicate that IDE may be involved in this regulation.

One of insulin's actions is to decrease protein degradation, thereby limiting the availability of amino acids for conversion to carbohydrate. We have shown that this effect is mediated, at least in part, through IDE [14]. We have reported that fatty acids can inhibit IDE and that the inhibition also results in a decrease in proteasome activity [16]. Fig. 1A shows that long-chain, but not medium-chain, FFA dose-dependently decrease proteasome activity. This is consistent with our in vitro observations [16]. This effect was seen at less than 100 μmol/L, a physiologically relevant concentration with respect to serum levels. Fig. 1B shows

that FFA and insulin have equivalent effects and are not additive. This suggests that the FFA and insulin may be working through the same mechanism.

In conclusion, previous in vivo studies have suggested that FFA can decrease protein degradation, although the mechanism has not been identified. We have shown that one possible mechanism is a decrease in proteasome activity, the major cellular protease. This mechanism may be mediated through IDE. This represents a possible mechanism for integrating nutrient and hormonal control of protein metabolism, with IDE being the common control point.

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