L-Leucine Activates Branched Chain \( \alpha \)-Keto Acid Dehydrogenase in Rat Adipose Tissue*

(Received for publication, November 17, 1980)

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The activity of branched chain \( \alpha \)-keto acid dehydrogenase in extracts of adipose tissue was elevated after homogenization of tissue segments which had been incubated in buffer containing 0.3 mM leucine. A maximum increase (4-fold) was observed in extracts of tissues incubated in buffer containing 2.5 mM leucine. \( \alpha \)-Ketoisocaproate and leucine caused maximum increases which were of similar magnitude and which required the same length of incubation of the tissue segments (5 to 15 min). The effect of leucine on branched chain \( \alpha \)-keto acid dehydrogenase activity was observed both in the presence and absence of insulin, which also increased the activity of the enzyme in tissue extracts.

Intact adipose tissue segments oxidized \([1-^{14}C]\)leucine at a maximum rate approximately 4 times that of \([1-^{14}C]\)valine. The rate of valine oxidation by intact tissue segments was doubled by addition of 0.2 to 0.5 mM unlabeled leucine, but not isoleucine, to medium containing 2 mM \([1-^{14}C]\)valine. Leucine, but not valine, also stimulated the rate of oxidation of 2 mM \([U-^{14}C]\)isoleucine by intact tissue segments. These results suggest that branched chain \( \alpha \)-keto acid dehydrogenase activity, which is thought to limit the rate of branched chain amino acid oxidation in adipose tissue, may be sensitive to changes in the concentration of leucine in rat blood.

While it has been known for some time that excess branched chain amino acids are degraded at extrahepatic sites (1–7), it is not yet clear which tissues account for the disposal of these amino acids when they are present in excess, nor is it known what signals control the degradation pathway. At least in the rat, muscle and adipose tissue appear to be the most important potential sites for degradation of branched chain amino acids (7). Degradation proceeds by transamination of the amino acids to form \( \alpha \)-keto acids, which are decarboxylated to their respective acyl-CoA derivatives by the mitochondrial branched chain \( \alpha \)-keto acid dehydrogenase complex. Insulin decreases the rate of branched chain amino acid oxidation in muscle (8) and increases it in adipose tissue (9, 10). While studying the effect of insulin on leucine metabolism in adipose tissue, we observed that, like insulin, leucine itself could increase the activity of branched chain \( \alpha \)-keto acid dehydr-

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1 These studies were supported by United States Public Health Service Grant AM21216 and by Postdoctoral Fellowship Award AM05542 (G. P. F.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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**RESULTS AND DISCUSSION**

Preincubation of adipose tissue segments in buffer containing leucine increased branched chain \( \alpha \)-keto acid dehydrogenase activity recoverable in fat-poor tissue extracts (Table I). Thus, the increased activity persisted even after 40-fold dilution of tissue water during homogenization. Although maximum stimulation (approximately 4-fold) required preincubation in buffer containing 2.5 mM leucine, a 50% increase in enzyme activity was observed when tissues incubated in 0.3 mM leucine were compared to tissues preincubated in buffer containing no leucine.

Leucine and \( \alpha \)-ketoisocaproate have similar effects on branched chain \( \alpha \)-keto acid dehydrogenase activity (Table II). Both compounds more than doubled enzyme activity after a preincubation period of 5 min and caused further increases which were observed after 15 min of preincubation. The similarity of their effects suggests that transamination of leucine to \( \alpha \)-ketoisocaproate is not the rate-limiting process involved in the activation of branched chain \( \alpha \)-keto acid dehydrogenase, or transamination may not even be required. Uptake of leucine or \( \alpha \)-ketoisocaproate by the cell or by mitochondria might be responsible for the slowness of the observed time course of branched chain \( \alpha \)-keto acid dehydro-

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* Some of the results in the present communication were presented earlier in preliminary form (11).
Activity of branched chain \(\alpha\)-keto acid dehydrogenase in extracts of adipose tissue after preincubation of tissue in buffer containing leucine

Tissue segments (350 to 500 mg) were incubated in 1 ml of Krebs-Ringer bicarbonate buffer for 15 min, transferred to a second vial containing buffer plus leucine for 1 h, homogenized, and centrifuged to obtain a fat-poor extract. Branched chain \(\alpha\)-keto acid dehydrogenase (BCDH) activity was measured as described under "Materials and Methods" in the presence of 200 \(\mu\)M \(\alpha\)-ketos[\(^{14}\)C]isocaproate. Each assay contained approximately 0.5 mg of protein derived from adipose tissue and represented the enzyme recovered from approximately 50 mg of tissue. Results are mean ± S.E.; 8 observations.

Table I

| Preincubation conditions | BCDH activity | p  |
|--------------------------|---------------|---|
| None                     | 0.390 ± 0.056 |   |
| 0.3 mM Leucine           | 0.630 ± 0.030 | <0.01 |
| 2.5 mM Leucine           | 1.69 ± 0.11   | <0.01 |
| 10 mM Leucine            | 1.96 ± 0.18   | <0.01 |

The increase relative to the 5 min result is also significant \((p < 0.01)\).

Leucine affects branched chain \(\alpha\)-keto acid dehydrogenase activation, or perhaps activation of the enzyme is indirect, involving some other process. After a 1-h preincubation with leucine and/or insulin, it was possible to compare their effects (Table III). When assayed at a low substrate concentration (22 \(\mu\)M \(\alpha\)-ketos[\(^{14}\)C]isocaproate), preincubation with insulin alone caused a 2.5-fold increase in branched chain \(\alpha\)-keto acid dehydrogenase activity, whereas leucine alone caused a 9-fold increase in enzyme activity. Addition of insulin and leucine to the preincubation buffer caused a further increase over that observed with either agent alone. No significant increase in enzyme activity was observed due to addition of insulin to the preincubation buffer when the enzyme was assayed in a reaction mixture containing 200 \(\mu\)M \(\alpha\)-ketos[\(^{14}\)C]isocaprate, either in the absence or presence of leucine (results not shown). This confirms our earlier study (10) and is consistent with the suggestion that insulin reduces the apparent \(K_m\) of branched chain \(\alpha\)-keto acid dehydrogenase for \(\alpha\)-ketosisocaproate from 100 to 30 \(\mu\)M (10). The effect of leucine was observed at substrate concentrations well above the \(K_m\) and reflects a change in \(V_{max}\).

No significant activation of branched chain \(\alpha\)-keto acid dehydrogenase was evident in tissue extracts after preincubation of tissue segments with insulin for 5 or 15 min (results not shown). Indeed, this observation confirms results obtained with intact tissue segments, where a 20-min lag occurred before insulin increased \(^{14}\)CO\(_2\) production from L-[\(^{14}\)C]leucine (9).

To evaluate the possibility that preincubation of tissues with leucine provides substrate stabilization of branched chain \(\alpha\)-keto acid dehydrogenase rather than activation of the enzyme, we next sought to determine whether leucine activates branched chain \(\alpha\)-keto acid dehydrogenase in situ. To this end, we compared the rates of \(^{14}\)CO\(_2\) production by intact tissue segments incubated with 1-\(^{14}\)C-labeled leucine or valine. Leucine was oxidized 3 to 6 times as rapidly as valine at all substrate concentrations (Fig. 1). These results are consistent with previous reports concerning the relative rates of leucine and valine oxidation (7, 12), although the maximum rates in Fig. 1 greatly exceed those observed previously at relatively low amino acid concentrations. The low rate of valine oxidation by tissue segments might be due to 1) slow uptake or transamination of valine (12), or 2) a smaller stimulation by valine than by leucine of branched chain \(\alpha\)-keto acid dehydrogenase activity, or both.

Adipose tissue extracts oxidized \(\alpha\)-ketoisovalerate, the \(\alpha\)-ketoc acid derived from valine, at a maximum rate of 11 milliunits/g of tissue, and \(\alpha\)-ketosisocaprate at a maximum rate of 6 mU/g tissue (10). These rates exceed those observed for oxidation of [\(^{1}\)\(^{14}\)C]valine, but not those observed for oxidation of [\(^{1}\)\(^{14}\)C]leucine in Fig. 1. Procedures not optimized for recovery of branched chain \(\alpha\)-keto acid dehydrogenase.

Table II

Activity of branched chain \(\alpha\)-keto acid dehydrogenase in extracts of tissues preincubated with leucine or \(\alpha\)-ketoisocaprate

Tissue segments were incubated in Krebs-Ringer bicarbonate buffer and transferred to vials containing indicated additions for a second incubation lasting 5 or 15 min. Tissue extracts were assayed for branched chain \(\alpha\)-keto acid dehydrogenase (BCDH) in the presence of 200 \(\mu\)M \(\alpha\)-ketos[\(^{14}\)C]isocaproate. The branched chain \(\alpha\)-keto acid dehydrogenase activity reported here is similar to that reported in Table I (note units). Results are means ± S.E.; 8 observations.

Table III

Effect of leucine and insulin on branched chain \(\alpha\)-keto acid dehydrogenase activity in adipose tissue extracts

Tissue segments were incubated in Krebs-Ringer bicarbonate buffer for 15 min and then transferred to vials containing additions as indicated for 1 h. Tissue extracts were assayed in the presence of 22 \(\mu\)M \(\alpha\)-ketos[\(^{14}\)C]isocaprate. Results are means ± S.E.; 8 observations.

Preincubation conditions | BCDH activity | p  |
|--------------------------|---------------|---|
| Control                  | 34.7 ± 7.7    |   |
| 2.5 mM Leucine, 5 min   | 122 ± 20      | <0.01 |
| 2.5 mM Leucine, 15 min  | 208 ± 16      | <0.001* |

* The increase relative to the 5 min result is also significant \((p < 0.01)\).

Fig. 1. Oxidation of leucine or valine by adipose tissue segments. Tissue segments (30 to 70 mg) were incubated for 30 min in 1 ml of Krebs-Ringer bicarbonate buffer containing [\(^{1}\)\(^{14}\)C]leucine or [\(^{1}\)\(^{14}\)C]valine (specific activity 33 to 470 \(\mu\)Ci/mmol). Each point represents the average rate of \(^{14}\)CO\(_2\) production during the incubation. Results are means ± S.E.; 8 observations.
a-keto acid dehydrogenase activity was tested by measuring buffer containing unlabeled leucine or isoleucine and 2 mM [l-
regulation of branched chain a-keto acid dehydrogenase will tissue, more than twice the maximum rate observed in Fig. 1,
branched chain a-keto acid dehydrogenase, and this was acid might be expected to decrease the apparent rate of branch-
oxidation of [l-14C]valine by adding unlabeled a-keto acid to
[FIG. 2. Effect of unlabeled leucine or isoleucine on the ox-
Unlabeled amino acid concentration, mM

Fig. 2. Effect of unlabeled leucine or isoleucine on the oxida-
tion of [1-14C]valine by adipose tissue segments. Tissue seg-
ments were incubated for 30 min in 1 ml of Krebs-Ringer bicarbon-
ate buffer containing 2 mM [1-14C]valine (71 μCi/mmoll) plus unlabeled
leucine or isoleucine, as indicated. Each point represents the average
rate of 14C-labeled lipid and 14CO2, was also stimulated by 0.5 mM unlabeled leucine but not by 0.5 mM unlabeled valine
(results not shown). The steep slope of the curve showing the rate of oxidation of [1-14C]leucine at low substrate concentra-
tions (Fig. 1) is also consistent with stimulation of branched chain a-keto acid dehydrogenase activity by low concentra-
tions of leucine. Thus, the rate of oxidation of 14C-labeled branched chain amino acids by tissue segments appears to
reflect the state of activation of branched chain a-keto acid dehydrogenase, and the enzyme is acutely sensitive to fluctuations in the concentration of leucine in the range found in rat blood plasma (13).

These results suggest that leucine may act as a physiological signal to regulate the activity of branched chain a-keto acid dehydrogenase in adipose tissue. A possible mechanism for the action of leucine is suggested by the observations of Hughes and Halestrap (14) that α-ketoisocaproatate prevents phosphorylation of a 49,000-dalton protein which appears to
be a subunit of branched chain a-keto acid dehydrogenase. This finding further suggests that the mechanism by which branched chain a-keto acid dehydrogenase is regulated may be similar to that of pyruvate dehydrogenase, which catalyzes an analogous reaction and which is activated by its substrate (15).

Acknowledgment—The technical assistance of Mr. Leonard Waice is gratefully acknowledged.

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