This document only includes an excerpt of the corresponding thesis or dissertation. To request a digital scan of the full text, please contact the Ruth Lilly Medical Library's Interlibrary Loan Department (rlmlill@iu.edu).

STUDIES ON THE REGULATION OF LEUCINE CATABOLISM IN LIVER AND HEART

Mechanism Responsible for Dichloroacetate Stimulation of Leucine Oxidation

by

Ronald Morrison Sans

Submitted to the Faculty of the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biochemistry, Indiana University

June 1979

Acceptance of this dissertation has been approved by:

Dr. Robert A. Harris — Robert A. Harris

Dr. David W. Allmann — David W. Allmann

Dr. Walter W. Jolly — Walter W. Jolly

Dr. Leon K. Knoebel — Leon K. Knoebel

Supervisory Committee

Dr. Robert A. Harris	Biochemistry (Chairman)
Dr. David. W. Allmann	Biochemistry
Dr. Walter W. Jolly	Biochemistry-Surgery
Dr. Leon K. Knoebel	Physiology

Major: Biochemistry

Minor: Physiology

Table of Contents

Abstract

I. Introduction.. 1

A. Literature Review.. 3

1. Introduction... 3

2. Leucine transamination................................. 3

3. α-Ketoisocaproate oxidation........................... 4

4. Dietary influence on leucine metabolism............ 7

5. Leucine oxidation in diabetes and starvation....... 8

6. Effect of dichloroacetate on metabolic processes... 9

7. References... 14

II. Studies on the regulation of leucine catabolism. Mechanism responsible for dichloroacetate stimulation of leucine oxidation by the liver.. 19

A. Abstract... 19

B. Introduction... 20

C. Materials and Methods.................................... 20

1. Isolation and incubation of liver cells............ 20

2. Assay of α-ketoisocaproate dehydrogenase, pyruvate dehydrogenase and leucine transaminase............... 21

3. Sources of materials.................................. 22

D. Results.. 24

1. The effect of dichloroacetate and pyruvate on various enzyme activities of leucine catabolism in homogenates of hepatocytes.............................. 24

2. Conversion of [2-^{14}C]dichloroacetate to [2-^{14}C]-glycine by isolated hepatocytes..................... 26

3. Characteristics of leucine transaminases of rat liver... 29

4. Characteristics of isoleucine and valine trans-aminases of rat liver................................... 34

E. Discussion... 34

F. References... 48

III. Studies on the regulation of leucine catabolism. Effects of dichloroacetate and 2-chloropropionate on leucine oxidation by the heart.. 50

A. Abstract.. 50

B. Introduction.................................... 51

C. Materials and Methods........................... 51

1. Perfusion techniques............................ 51

2. Analytical methods.............................. 53

3. Sources of material............................. 53

D. Results... 53

1. The effect of dichloroacetate on leucine oxidation by perfused rat heart............................. 53

2. The effect of dichloroacetate on α-ketoisocaproate oxidation by perfused rat heart...................... 56

3. The effects of 2-chloropropionate on leucine and α-ketoisocaproate oxidation in the perfused rat heart. 60

4. The effects of dichloroacetate and 2-chloropropionate on acetate and isovalerate oxidation by the perfused rat heart.. 60

5. The effect of dichloroacetate on the pyruvate concentration in the heart and perfusion medium....... 63

6. The effect of lactate and pyruvate on leucine oxidation by the perfused rat heart.................. 63

E. Discussion...................................... 65

F. References...................................... 72

IV. Studies on the regulation of leucine catabolism. Mechanism responsible for dichloroacetate stimulation of leucine oxidation by the heart.................................... 74

A. Abstract.. 74

B. Introduction.................................... 75

C. Materials and Methods........................... 76

1. Perfused heart preparation and isolation of heart mitochondria..................................... 76

2. Measurements of rates of substrate oxidation in the perfused heart and intact mitochondria........... 76

3. Extraction, assay and activation of the α-keto-isocaproate dehydrogenase complex from isolated heart mitochondria..................................... 77

4. Sources of materials.............................. 78
D. Results.. 79
1. Effect of various substrates and dichloroacetate on the oxidation of [1-^{14}C]leucine by the perfused rat heart.. 79
2. Activity of the α-ketoisocaproate dehydrogenase complex of mitochondria isolated from hearts perfused with various substrates and dichloroacetate........... 83
3. The effect of glucose and dichloroacetate on [3-^{14}C]-β-hydroxybutyrate oxidation by the perfused rat heart. 89
4. Effects of various substrates and dichloroacetate on the oxidation of [1-^{14}C]α-ketoisocaproate by rat heart mitochondria.................................... 91
E. Discussion.. 97
F. References.. 105
V. Appendix.. 107
VI. Curriculum vitae.. 113

ABSTRACT

Leucine, an essential amino acid, is important as a substrate for protein, fatty acid, and sterol biosynthesis, as a source of energy, and as a regulator of several metabolic processes. Insulin secretion, urea formation, and glucose oxidation are among the processes that are affected by leucine. The purpose of this study was to learn more about the control of leucine catabolism, a major determinant of leucine availability. The study makes use of isolated hepatocytes, liver homogenates, perfused heart, isolated mitochondria, and extracts of heart mitochondria. Dichloroacetate, a known activator of the pyruvate dehydrogenase complex and stimulator of leucine oxidation in liver, was found in this study to stimulate leucine oxidation by the heart. The mechanisms responsible for the actions of dichloroacetate on leucine catabolism have been established for both liver and heart. Surprisingly, dichloroacetate has different mechanisms of action in these two tissues. Elucidation of the mechanism of action of dichloroacetate on leucine catabolism has made a significant contribution to the understanding of the regulation of leucine catabolism. The α-ketoisocaproate dehydrogenase complex, which catalyzes the second step of leucine catabolism and is analogous in many ways to the pyruvate dehydrogenase complex, was not stimulated by dichloroacetate in liver. Rather, dichloroacetate is dechlorinated by liver cells to form glyoxylate which promotes leucine catabolism by serving as an amino group acceptor for either direct or indirect transamination of leucine. Support for this mechanism includes the following: (a) amino acids which serve as substrates for glyoxylate aminotransferase

block the stimulatory effect of dichloroacetate on leucine oxidation; (b) glyoxylate stimulates oxidation of leucine; (c) both dichloroacetate and glyoxylate increase the glycine content of isolated hepatocytes; (d) [2-^{14}C]dichloroacetate is converted by isolated hepatocytes to [^{14}C]glyoxylate, [^{14}C]oxalate, and $^{14}CO_2$; and (e) 2-chlorpropionate, which also activates the pyruvate dehydrogenase complex but is not converted to glyoxylate, does not stimulate leucine oxidation. Thus in liver, in contrast to its mechanism of action on pyruvate metabolism, dichloroacetate promotes leucine catabolism by formation of glyoxylate which serves as substrate for the transamination of leucine by glyoxylate aminotransferase. Dichloroacetate also promotes leucine oxidation by heart, but unlike liver, the site is at the α-ketoisocaproate dehydrogenase complex. This conclusion is based on the observation that dichloroacetate increases L-[1-^{14}C]leucine oxidation to $^{14}CO_2$ but decreases the steady state concentration of α-ketoisocaproate. It also increases the oxidation of [1-^{14}C]α-ketoisocaproate but not that of [1-^{14}C]isovalerate. 2-Chloropropionic acid also stimulates leucine oxidation in heart, and thus it follows that activation of leucine oxidation in heart by these activators of pyruvate dehydrogenase is not explained by glyoxylate formation. Furthermore,in the absence of any other oxidizable substrate,the perfused heart oxidizes [1-^{14}C]leucine to $^{14}CO_2$ at a significant rate and releases only negligible amounts of [1-^{14}C]α-ketoisocaproate into the perfusion medium. The α-ketoisocaproate dehydrogenase complex assayed in extracts of mitochondria prepared from such perfused hearts was very active. Under such perfusion conditions, dichloroacetate had almost no effect on [1-^{14}C]leucine oxidation, [1-^{14}C]α-ketoisocaproate release, or α-keto-

isocaproate dehydrogenase activity. Perfusion of the heart with some other oxidizable substrate, e.g. glucose, pyruvate, or ketone bodies, resulted in an inhibition of [1-^{14}C]leucine oxidation to $^{14}CO_2$ and the release of large amounts of [1-^{14}C]α-ketoisocaproate into the perfusion medium. The α-ketoisocaproate dehydrogenase complex, assayed in extracts of mitochondria prepared from such hearts, was almost completely inactivated. The enzyme could be re-activated, however, by incubating the mitochondria at 30°C without an oxidizable substrate. Under these perfusion conditions, dichloroacetate greatly activated [1-^{14}C]leucine oxidation, decreased [1-^{14}C]α-ketoisocaproate release into the perfusion medium, and activated the α-ketoisocaproate dehydrogenase complex. It is concluded that leucine oxidation by heart is regulated by the activity of the α-ketoisocaproate dehydrogenase complex which is subject to interconversion between active and inactive forms. Oxidizable substrates establish conditions which inactivate the enzyme. Dichloroacetate, known to activate the pyruvate dehydrogenase complex by inhibition of pyruvate dehydrogenase kinase, also activates the α-ketoisocaproate dehydrogenase complex. This provides strong, albeit indirect, evidence that the α-ketoisocaproate dehydrogenase complex of heart tissue is subject to regulation by interconversion between inactive and active forms by the process of phosphorylation and dephosphorylation.