Appendix C – Detailed Research on HCA
Section I: Basic Mechanisms of Action

Overview

Whenever the rate of glycolysis exceeds energy requirements and/or the capacity to produce and store glycogen, the resulting acetyl-CoA units derived from carbohydrates (and under some conditions, also proteins) are turned into fatty acids and cholesterol at the first step of the Krebs Cycle. Acetyl-CoA, the primary substrate for fatty acid biosynthesis, is a product of pyruvate oxidation within the mitochondrion when demands for energy in the form of ATP are low. However, fatty acid biosynthesis does not take place within the mitochondrion, but instead occurs within the cytosol of the cell, and acetyl-CoA cannot cross the mitochondrial membrane to accomplish this synthesis. Acetyl-CoA can cross the membrane only as citrate, and therefore the excess acetyl-CoA within the mitochondrion is exported in this form. In the cytosol, ATP-citrate lyase is required to cleave citrate in the above reaction to release acetyl-CoA. HCA works by acting as a competitive inhibitor of the enzyme ATP-citrate lyase within the following reaction pathway of fatty acid biosynthesis in the presence of the divalent ion of magnesium:

\[
\text{ATP + citrate + CoA} \rightarrow \text{acetyl-CoA + oxaloacetate + ADP + Pi}
\]

where ATP = adenosine triphosphate, CoA = coenzyme A, ADP = adenosine diphosphate and Pi = orthophosphate ion. Citrate is synthesized from oxaloacetate and acetate.

The full effects of HCA reflect the extensive role of citrate in the metabolism of carbohydrates and the synthesis of lipids. Citrate does more than merely ferry acetyl units from the mitochondrion into the cytosol. Once in the cytosol, citrate itself stimulates the fatty acid synthetic pathway through its activation of acetyl-CoA carboxylase. At the same time, it also serves to regulate glycolysis at three points. Citrate inhibits both phosphofructokinase and pyruvate dehydrogenase. Likewise, the presence of excess citrate at the point at which acetyl-CoA enters the Krebs Cycle inhibits the enzyme citrate synthase from producing additional citrate.

The effects of HCA within this framework are complex. Through its basic action of inhibiting ATP-citrate lyase, HCA causes an increase in the extramitochondrial pool of citrate, and thereby also in an increase in the inhibition of glycolysis at the regulatory junctures. HCA itself may mimic some of the regulatory activities of citrate. Three secondary effects thus appear with the administration of HCA. First, the production of fatty acids and cholesterol is reduced under most conditions. Second, the glycolytic pathway is slowed. Third, carbon units are redirected toward glycogen production. The following figures illustrate these points.
The Krebs/Citric Acid Cycle in Fatty Acid Synthesis from Glucose
Partial Regulation of Glycolysis (Metabolism of Carbohydrates) and the Krebs Cycle by HCA

**GLYCOLYSIS**
- Glucose → Glucose-6-phosphate
  - Glucose-1-phosphate → Fructose-6-phosphate
  - Fructose-1,6-bisphosphate

**Glycogen Production**

**Pyruvate**
- Pyruvate kinase (ATP Blocks)

**KREBS CYCLE**
- Oxaloacetate → Citrate
- Citrate → α-Ketoglutarate
- α-Ketoglutarate → Succinyl CoA
- Succinyl CoA → Succinate
- Fumarate → Malate

**NADH Blocks**

**Fatty Acids**
- Citrate lyase
- Isocitrate

**Odd chain Fatty Acids**
- Oxaloacetate
- α-Ketoglutarate
- Succinyl CoA
- Amino Acids

**ATP & Citrate Enzyme**
- ATP Blocks
Possible Pathways of Acetyl-CoA Across the Mitochondrial Membrane
HCA Inhibits ATP-Citrate Lyase

**In Vitro Study:**

"...a relatively powerful inhibition of citrate cleavage is observed in the presence of (-)-hydroxycitrate. The stereoselectivity of the inhibition of citrate cleavage by hydroxycitrate...shows that (-)-hydroxycitrate is a much more powerful inhibitor than (+)-allo-hydroxycitrate."


**In Vitro Study:**

"There are three citrate enzymes which catalyze the same bond-making and -breaking reaction which involves the equilibrium of citrate with oxalacetate and an acetol moiety....(4S)-OHcit-(p,n) [(-)hydroxycitrate] is a very potent linear competitive inhibitor of ATP-citrate lyase from rat liver...the other stereoisomer with a [sic] hydroxyl group substituted on carbon 4, was a less effective inhibitor, but a more potent inhibitor than the two isomers with hydroxyl groups substituted on carbon 2....The four hydroxycitrates bind well and all undergo a reaction except (4S)-OHcit-(p,n) [(-)hydroxycitrate] which is found to be an extremely potent binder."


**Review Article:**

"In the breakdown of foodstuffs, pyruvate derived from carbohydrate and fatty acids derived from fat are converted to acetyl-CoA by the intramitochondrial enzyme systems. Under normal conditions of carbohydrate utilization, the rate of oxidation of the acetyl group of acetyl-CoA via the citric acid cycle is determined by the energy demands of tissue, or its equivalent, the availability of ADP. When the carbohydrate intake of an animal is in excess of its energy requirements the glycogen stores become filled. Thereafter excess carbohydrate is broken down to pyruvate. The reactions of glycolysis occur in the extramitochondrial space of the cell. However, oxidation of pyruvate to acetyl-CoA occurs in the mitochondria. Acetyl groups not required for energy production are converted into fatty acids. In the rat, fatty acid synthesis occurs predominantly in the extramitochondrial space of the cell [i.e., the cytosol]. The transfer of the acetyl group of acetyl-CoA from the intramitochondrial space into the cytoplasm is thus an important step in the conversion of carbohydrate into fat by non-ruminant mammals."

**HCA Does Not Inhibit Synthesis from Acetate, a Minor Pathway**

**In Vitro Study:**

"In isolated hepatocytes it [HCA] inhibits fatty acid synthesis from glucose, but it does not affect fatty acid synthesis from acetate."


**Perfusion Experiment:**

"In this paper an inhibition of hepatic cholesterol and fatty acid synthesis by (-)-hydroxycitrate in the isolated perfused rat liver is reported; acetate reversed the effect on fatty acid synthesis."


**Perfusion Experiment:**

"In the presence of (-)-hydroxycitrate, translocation of acetyl units via systems other than citrate or acetoacetate contributes 78 and 20% of the total carbon incorporated into fatty acids and sterols, respectively....It follows that the pathways not involving citrate or acetoacetate could contribute only 15 and 5% of the supply of carbon for fatty acid and sterol synthesis. Our data confirm that the citrate cleavage pathway is the predominant form of acetyl translocation in rat liver."

Articles


