Effect of (−)-Hydroxycitrate on Fatty Acid Synthesis by Rat Liver in Vivo*

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SUMMARY

Incorporation of \(^{1}H\) from \(^{2}H\)O was used to measure the rate of fatty acid synthesis in rat liver. (−)-Hydroxycitrate strongly inhibits fatty acid synthesis in vivo. Fatty acid synthesis was measured by the incorporation of tritium from water.

It was shown previously that (−)-hydroxycitrate\(^{1}\) is a powerful inhibitor of citrate cleavage enzyme (1). The present paper shows that (−)-hydroxycitrate is a highly effective inhibitor of fatty acid synthesis by rat liver in vivo. Fatty acid synthesis was measured by the incorporation of tritium from water.

EXPERIMENTAL PROCEDURE

Methods—Rats were obtained from the Charles River Breeding Laboratories, North Wilmington, Massachusetts. Upon receipt the animals were placed on a scheduled diet consisting of Purina Laboratory chow (0.5-inch Checkers), Ralston Purina Company, St. Louis, Missouri, given to one animal per cage, from 9 a.m. to 12 noon. Water was made available ad libitum. The animals were kept in an artificially lighted room, with the light on from 0 a.m. to 6 p.m. The room temperature was 24°. Although rats are normally nocturnal feeders, the above schedule yielded very reproducible rates of fatty acid synthesis. I am indebted to Dr. O. Neal Miller for informing me in advance of publishing his own work that this regimen leads to much more reproducible rates of fatty acid synthesis than are obtained when the animals receive food ad libitum. After 7 to 10 days on the chow diet the rats were fasted for 48 hours—that is to say, they missed one scheduled feeding—and they were then fed a scheduled diet high in fructose or glucose (2) for 10 to 15 days. Weights of the animals at the time of use are indicated in the legends.

Unless otherwise indicated, experiments with the rats were started 3.5 to 4 hours after the animals started their last feeding schedule. When indicated rats were injected with 2 to 20 mm sodium (−)-hydroxycitrate, pH about 7.4, in amounts indicated in the legends. Controls received an equal volume of isotonic NaCl. Most of these injections were given intraperitoneally, but some administrations were made into the tail vein for comparison. After 45 min each rat was given 0.2 ml of a solution containing about 1 mCi of \(^{3}H\)O by injection into a tail vein. The solution had a pH of about 7.4, and was made approximately isotonic by addition of sodium chloride. The rats were killed by decapitation 45 or 60 min after administering \(^{3}H\)O, blood was collected, and the liver was excised quickly and weighed. Immediately thereafter the liver was subjected to one of two treatments. It was either dropped into 19 ml per g of liver of chloroform-methanol (3:1, by volume) and homogenized with a Sorvall Omni-Mixer homogenizer (an overhead type of blender), or it was wrapped into aluminum foil and pressed quickly between two blocks of Dry Ice. In the latter case the liver was stored at −20° and homogenized in chloroform-methanol at a later date. Duplicates of either 10 or 20 ml of the clear chloroform-methanol extract were evaporated to dryness and saponified with 2 ml of 5 N NaOH at 90° for 2 hours. Acidification and extraction were carried out as described previously (3). The extracted fatty acids were counted in 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene in toluene. The specific radioactivity of the body water of each animal was determined by counting a suitably diluted aliquot of its plasma. Aqueous samples were counted in the scintillator described by Bray (4); the results so obtained were converted to the all toluene scintillator mentioned above. In several experiments the radioactivity of the nonsaponifiable fraction was examined. It contained less than 5% of the total radioactivity incorporated into fatty acids.

The \(^{2}H\)O method for studying fatty acid synthesis described above is based on work of Fain and Scow (5). According to Jungas (6), the \(^{2}H\)O method yields an average of 0.87 atom of \(^{1}H\) incorporated per carbon atom incorporated into palmitate. The isotope effects, exchange reactions, and the metabolic pathways which lead to this particular fraction have been discussed in detail by Jungas (6), and need not be repeated here. Suffice it to say that the isotope effects and exchange reactions which occur are approximately constant and are of practical consequence to the present paper. In what follows results are expressed as micromoles of \(^{2}H\)O incorporated into long chain fatty acid per g of fresh weight of liver per hour. This can be converted to micromoles of acetyl group incorporated by dividing by 0.87 (or multiplying by 1.15).

Estimates of rates of fatty acid synthesis in vivo have been obtained in a number of ways. Most commonly, the rate of incorporation of a labeled precursor such as \(^{1}C\)-acetate or \(^{1}C\)-acetate into fatty acids is measured directly. The present paper describes a method for measuring rates of fatty acid synthesis in vivo that is advantages in that the rate of synthesis can be measured by the incorporation of even small amounts of water labeled with a single atom of \(^{1}H\) into long chain fatty acid per g of fresh weight of liver per hour. This can be converted to micromoles of acetyl group incorporated by dividing by 0.87 (or multiplying by 1.15).

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FIG. 1. Fatty acid synthesis by rat liver in vivo as a function of time. The animals received a scheduled diet high in glucose for 10 days as described under "Methods". The weight of the animals at the time of use was 148.2 g (S.E. = ±5.6, number of animals = 10). The results shown are from a single experiment. Each rat received 0.2 ml of 3H2O in isotonic NaCl solution into a tail vein 3.5 to 5 hours after starting the last feeding schedule.

FIG. 2. Fatty acid synthesis by rat liver in vivo in relation to the start of feeding. The animals received a scheduled diet high in fructose as described under "Methods". The weight of the animals at the time of use was 87.4 g (S.E. = ±1.64, number of animals = 39). The results shown were obtained in four separate experiments over a period of 20 days. The points show the mean ±S.E. The number of animals in each group is in parentheses. (The points at 1 hour show the results obtained with two animals and the mean.)

FIG. 3. Inhibition of fatty acid synthesis by (-)-hydroxycitrate. The inhibitor was given intraperitoneally 45 min before injecting 3H2O. Animals received a diet high in glucose (●) or fructose (▲) as described under "Methods". The weight of the animals at the time of use was 158.8 g (S.E. = ±4.3, number of animals = 21) on the diets high in glucose and fructose, respectively. The points show the mean ±S.E. The number of animals per point is in parentheses. (The point (●) at 1.53 mmoles of hydroxycitrate per kg of rat represents a single animal; the points (▲) at 4.0 mmoles of hydroxycitrate per kg of rat represent two rats and the mean.)
The shown arc from the separate experiments. The points show the to 300/ This is equivalent to about 2.9 mg of hydr0xycitrate intravenously within 30 to 60 sec. The first given (-)-hydroxycitrate intraperitoneally and then SH2O, which received inhibitor and 3H2O at zero time, the animals were in the case of animals in normal, but that it must then have decreased rapidly as the inhibitor was taken up by the liver cells. The longest interval between administering hydroxycitrate and starting the measure-

ment of fatty acid synthesis shown in Fig. 4 was 50 min. Here too, the measurement of the rate of fatty acid synthesis lasted for 45 min. This means that the inhibition of fatty acid synthesis remained strong between 50 and 95 min after administering hydroxycitrate. Four additional experiments also showed that the inhibition of fatty acid synthesis remains strong 2 to 3 hours after giving hydroxycitrate.

**DISCUSSION**

(-)-Hydroxycitrate is a very strong inhibitor of citrate cleavage enzyme (1) and of fatty acid synthesis in vivo (10). The present paper shows that (-)-hydroxycitrate is also an excellent inhibitor of fatty acid synthesis in vivo.

The Ki of (-)-hydroxycitrate in the reaction catalyzed by citrate cleavage enzyme is between 0.2 and 0.6 \( \mu \)M depending on conditions (1). The amount of (-)-hydroxycitrate required for 50% inhibition of fatty acid synthesis in vivo, namely 0.28 mmole per kg of body weight, is roughly 700 times larger than the \( K_i \) for citrate-cleavage enzyme. It seems likely that one of the factors limiting the effectiveness of (-)-hydroxycitrate in vivo is its passage across the cell membrane.

A possibility which was discussed previously (10), and which should not be overlooked here, is that the inhibition of citrate cleavage enzyme by (-)-hydroxycitrate in vivo switches on a pathway for the generation of extramitochondrial acetyl-CoA which does not involve citrate. Such a pathway would circumvent the inhibited citrate cleavage reaction and this would manifest itself as an apparently lower effectiveness of (-)-hydroxycitrate than might otherwise be expected. There is at present no evidence favoring such an alternate pathway in non-ruminant mammals.

Another possibility which should be considered is that (-)-hydroxycitrate inhibits the transfer of citrate from the mitochondrial matrix into the cytoplasm. Experiments in which the excretion of citrate formed in liver mitochondria was measured in the presence and absence of (-)-hydroxycitrate suggest that this type of inhibition does not occur, or if it occurs that it cannot be large (10). Measurements by Drs. G. R. Williams and B. H. Robinson show that (-)-hydroxycitrate does not exchange with intramitochondrial citrate, and that it does not inhibit the egress of citrate from mitochondria previously loaded with labeled citrate.

When the dose of (-)-hydroxycitrate was 1 mmole per kg of body weight the inhibition of fatty acid synthesis was about 75% (Fig. 3). The control rate of fatty acid synthesis was about 60 mmoles of H2O incorporated into fatty acids per g of liver per hour. This is equivalent to 69 mmoles of acetyl group incorporated per g of liver per hour, or 8 mmoles per liver per min (assuming the liver to weight 7 g). An inhibition of 75% of this rate corresponds to 6 mmoles of acetyl group per liver per min being prevented from going into fatty acids. In other words, this amount of carbon must be channelled into alternative products. The nature of these products is presently under investigation.

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