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CONVERSION OF MEVALONOLACTONE TO ITS OPEN-CHAIN SALT BY A SERUM ENZYME*

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SUMMARY. Mevalonic acid exists in two forms at physiologic pH: an open-chain salt and a cyclic lactone. Studies by others have demonstrated preferential utilization of the salt by the brain and of mevalonolactone by isolated hepatocytes.

The major finding of this report is the presence of a very active delta-lactonase in rat serum which rapidly converts mevalonolactone into the open-chain salt. Studies showed that the female rat oxidized 92 nmol of [5-^{14}C]mevalonic acid salt or lactone to CO_2 in 7 h, while the male oxidized only 52 nmol of either form of the injected mevalonate. There was no statistical difference in the in vivo metabolism of the salt or lactone. When mevalonolactone (0.1 mM) was incubated in neat rat serum for 20 sec, more than 82% of the lactone was converted to the salt as compared to 8% in heat-inactivated serum. These observations demonstrate for the first time the presence of a heat-labile delta-lactonase (possibly mevalonolactone hydrolase) in rat serum.

INTRODUCTION

Mevalonate, the product of the rate-limiting step of cholesterol synthesis, can exist in the body in two forms: the six-member cyclic lactone and the open-chain salt. Fumagalli et al. provided in vitro evidence of a differential uptake and utilization of mevalonolactone and potassium mevalonate in rat brain (2). They demonstrated that potassium mevalonate was incorporated into brain cholesterol more rapidly than mevalonolactone; however, liver slices metabolized mevalonolactone at a slightly higher rate. Later studies by Edwards et al. (3, 4) showed that rat hepatocytes converted mevalonolactone to cholesterol at rates six- to eightfold greater than that of the salt. These reports stimulated our investigation of preferential utilization by

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various tissues, because many of our recent studies of the two major pathways of mevalonate metabolism have used the open-chain salt exclusively (5-10).

The in vitro data cited above suggested that the physical form of mevalonate will determine the site and amount of utilization; however, in our present in vivo study we found no qualitative or quantitative differences between the metabolism of the salt and the lactone of mevalonate. Our findings suggested that injected mevalonate is converted to one, predominant molecular form in the bloodstream of the animal. This thesis was confirmed by in vitro studies demonstrating the existence, in rat serum, of a very active delta-lactonase, possibly mevalonolactone hydrolase, which is capable of rapidly breaking the lactone to the open-salt form.

EXPERIMENTAL PROCEDURES. Materials were purchased from the following vendors: (R,S)-[5-^{14}C]mevalonic acid sodium salt (15 mCi/mmol), from Research Products, Inc.; [1α,2α-^{3}H]cholesterol (43 Ci/mmol), from Amersham Radiochemical Centre; unlabeled (R,S)-mevalonolactone and inorganic reagents, from Sigma Chemical Co.; anion exchange resin AG 1-X8 (200-400 mesh), formate form, from Bio-Rad Laboratories. The thin-layer chromatography was carried out using Brinkmann Sil G plates. Radioautography was carried out on Kodak RP-14 x-ray film. The liquid scintillation fluids contained scintillation-grade toluene from Packard Corp., scintillation-grade 1,4-bis[2-(5-phenyloxazolyl)]benzene and 2,5-diphenyloxazole from Amersham/Searle, and Bio-Solv III and Triton X-100 from Beckman Instruments.

The aqueous solutions of (R,S)-[5-^{14}C]mevalonolactone and salt were prepared from the same batch of mevalonate; one was converted to the mevalonolactone and was stored frozen at a pH less than 3. The salt was stored frozen at a pH greater than 9. The final activity of the injected mevalonolactone for the in vivo studies was 5 μCi/1,046 nmol/0.4 ml.

In vivo studies. For the in vivo studies, male and female Sprague-Dawley rats weighing between 200 and 400 g were maintained on a reverse 12-h light cycle. The animals were anesthetized with diethyl ether between 8:30 and 9:30 A.M., and 0.4 ml of the labeled substrate was injected into the tail vein. The expired $^{14}CO_2$ was collected continuously in gas-washing bottles containing 1 N NaOH as described earlier (7). At the times noted, 0.2-ml samples of the NaOH solution were collected and counted on a Beckman liquid scintillation counter. After 7 h the rats were killed and the livers, kidneys and carcasses were saponified separately and analyzed for ^{14}C-lipids as described previously (6).

In vitro studies. For the in vitro studies, Sprague-Dawley rats were anesthetized and exsanguinated between 8:30 and 9:30 A.M. The pooled blood serum was incubated at 37°C with freshly purified [5-^{14}C]mevalonolactone. The reaction mixture was then rapidly cooled and analysis carried out at 4°C.

Analytical Methods. The mevalonolactone and mevalonate salt in the reaction mixture were rapidly analyzed by means of anion exchange chromatography. The anion exchange columns were prepared by suspending the AG 1-X8 resin in distilled water and adding it to Pasteur pipettes until the packed resin reached 5 cm in height. The columns were first flushed with 2-4 ml of

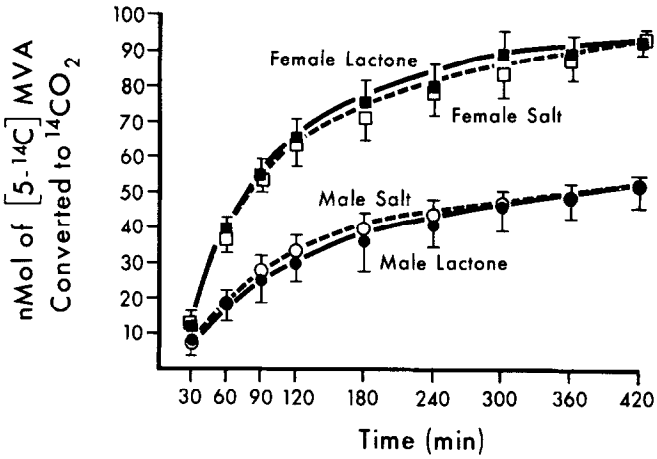

Fig. 1 Oxidation of [5-^{14}C]mevalonic salt and lactone to $^{14}CO_2$ in intact males and females. The final activity for both forms of mevalonate was 5 μCi/1.046 nmol/0.4 ml. The bars represent means ± S.E. See "Experimental Procedures" for details. ■ female, salt form, □ female, lactone form; ● male, salt form, O male, lactone form.

distilled water. The mevalonolactone was eluted with 1-2 ml of water. The columns with their retained radioactivity were then washed with 2 ml of 0.1 N NaCl and 1 ml of 0.2 N NaCl, following which the polar mevalonate metabolite was eluted with 2 ml of 0.5 N NaCl. For identification of mevalonic salt, tritiated mevalonate was added as an internal standard and marker for mevalonolactone. The eluant was then acidified with 10 N HCl to a final solution pH of 3. The mixture was left at 37°C for 15 min to lactonize any ^{14}C-mevalonic salt as well as the [^{3}H]mevalonate internal standard. The solution then was extracted with diethyl ether; the extract was dried, the residue dissolved in acetone, and a 200-μl aliquot was plated on a thin-layer chromatography plate. The plate was developed in acetone:benzene (1:1) and then radioautographed.

RESULTS

In vivo metabolism of mevalonic salt and lactone

Shunt pathway. The initial experiments were designed to determine any differences in metabolism of the mevalonate salt and lactone in the intact animal. As shown in Fig. 1, the three male animals injected with the lactone oxidized an average of 52.0 nmol/7 h to CO_2, a value equal to the 52.4 nmol obtained with the 3 males injected with the salt. The 3 female animals oxidized an average of 92.3 nmol of mevalonolactone/7 h, a value identical to that in the 3 females injected with mevalonate salt. There was therefore no difference in either sex in the metabolism of these two forms of mevalonic acid by the nonsterol (shunt) pathway. The entire (i.e., salt and lactone)

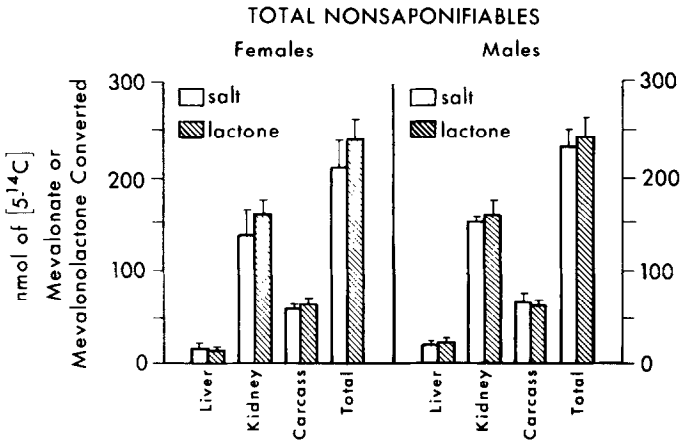

Fig. 2 Metabolism of [5-^{14}C]mevalonic acid and lactone to ^{14}C-labeled nonsaponifiable lipids in male and female rats. The bars represent means ± S.E. See "Experimental Procedures" for details.

group of males oxidized an average of 52.3 ± 3.1 nmol of mevalonic acid to CO_2, compared to the female average of 92.3 ± 1.6 nmol.

Sterol pathway. To further quantify any differences in the metabolism of mevalonate salt and lactone, we measured the sterol products synthesized in vivo from the two forms of mevalonate. As shown in Fig. 2, there was no significant difference in the in vivo metabolism of the two molecular forms of mevalonate in either sex.

In vitro conversion of mevalonolactone in rat serum. Since the in vivo data raised the possibility that the mevalonic acid is converted to the same molecular form prior to metabolism, the next step was to determine whether the salt or the lactone is predominantly present in the serum. The salt and lactone were incubated with the neat sera. Analysis by anion exchange chromatography showed that the mevalonate salt remained unchanged in the sera; however, the lactone form was rapidly converted to a more polar compound, presumably the mevalonate salt.

That the hydrolysis of the mevalonolactone was due to the presence of an enzyme in the rat serum was indicated by the finding that the sera, when diluted 1:6, converted 64% of the lactone to the salt in a 9-min period as compared to the 10% spontaneous hydrolysis in the heat-inactivated serum.

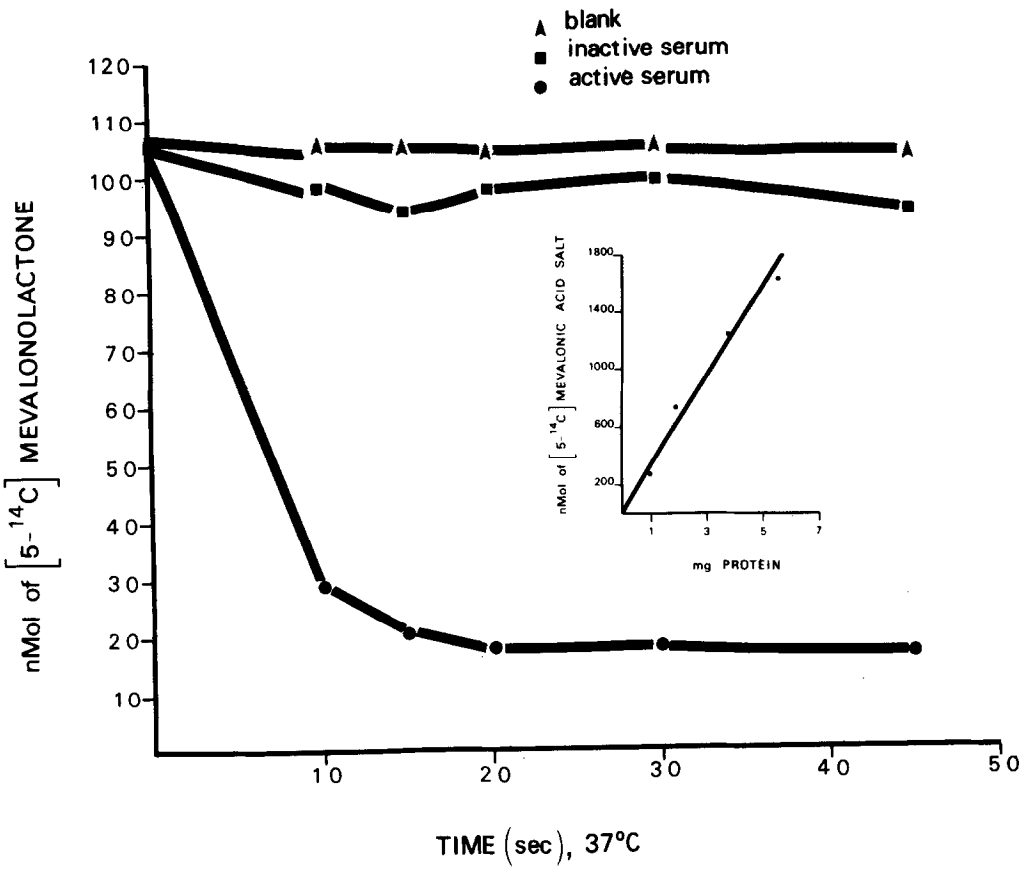

Fig. 3 Time course of mevalonolactone hydrolysis activity in active and heat-inactivated rat serum. Assay volume was 250 µl with a lactone concentration of 105 µM. Inset A Plot of hydrolysis activity with respect to protein concentration. The assay volume was 200 µl with a final lactone concentration of 11.7 mM. Assay was carried out at 37°C for 15 min.

Since the in vivo experiments involved the injection of 1,000 nmol of mevalonic acid, we next attempted to determine whether there is sufficient enzyme activity in serum to convert this amount of mevalonolactone rapidly to the salt. Assuming a blood volume in a 200-g rat to be 10 ml, with 1,000 nmol of substrate injected, the initial and highest concentration of substrate in vivo would then be 0.1 mM. When 0.1 mM mevalonolactone was incubated for 20 sec in fresh serum, on subsequent analysis 82% of the radioactive label was retained on the anion exchange column, as compared to 8% in the heat-inactivated serum (Fig. 3). This in vitro study would suggest that the rapid

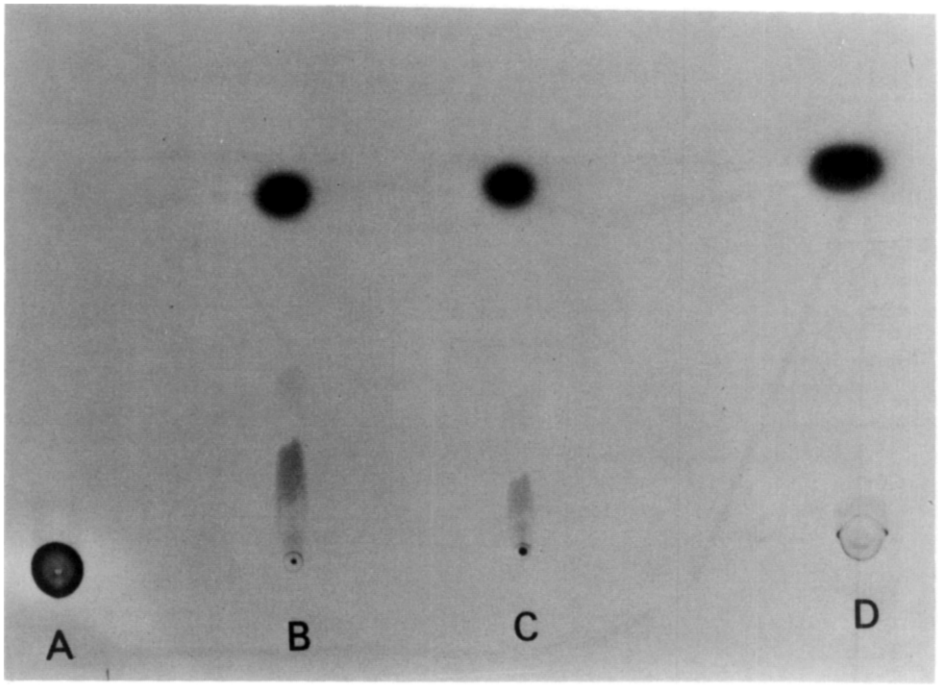

Fig. 4 Radioautograph of a thin-layer chromatogram of ^{14}C-mevalonate following incubation with rat serum and standard. A, mevalonic acid standard; B, product from assay; C, product from assay; D, mevalonolactone standard. R_f = 0.6. Original assay volume was 200 µl with a final lactone concentration of 3.68 mM. Assay time was 5 min at 37°C. Assay samples were pooled and analyzed as described in "Experimental Procedures."

conformational change of the lactone to the salt is complete within seconds after injection and is due to the presence of a delta-lactonase in the serum. As shown in inset A, the hydrolytic activity in serum was also linear with respect to protein concentration.

Theoretically, the retained moiety on the anion exchange column could be any metabolite of the lactone. As is shown in Fig. 4, the acidified, retained moiety had an R_f value identical to that of the authentic tritiated mevalonolactone internal standard and the ^{14}C-mevalonolactone plate standard. The [5-^{14}C]mevalonolactone converted to the polar species was fully recovered as the mevalonate on the TLC plate.

DISCUSSION

The major finding of this report is the presence in rat serum of a very active delta-lactonase that rapidly converts mevalonolactone into the corresponding open-chain salt of mevalonic acid. This study was stimulated initially by reports of preferential uptake and utilization of mevalonic salt by rat brain slices and of mevalonolactone by isolated hepatocytes (2-4). Previous studies from this laboratory had quantified the relative roles of the sterol and non-sterol (or shunt) pathways of the metabolism of mevalonate. Further, we had found that the female rat metabolizes mevalonate by the shunt pathway at a rate approximately twice that of the male (9). Since these conclusions were based on studies employing the open-chain form of mevalonate exclusively, it was important to determine whether the lactone and the salt of mevalonate were differentially metabolized under in vivo conditions. As shown in Fig. 1, the sex difference previously described in the shunt pathway was readily demonstrated with both the lactone and salt forms of mevalonic acid. Further, no statistical difference was observed in the oxidation of the two configurations of mevalonate to CO_2. Likewise, no statistical differences were observed in the synthesis of nonsaponifiable lipids from the salt and lactone forms in any tissue.

The discrepancy between our in vivo results and those of the published literature describing in vitro preferential uptake of the two forms of mevalonate (2-4) could best be explained by an enzymatic conversion of the mevalonate to one, predominant form in the bloodstream of the injected animal. This hypothesis was tested directly by adding trace amounts of both the salt and lactone forms of mevalonic acid to isolated rat serum. The salt remained unchanged, but the lactone was converted to the salt form within seconds of addition.

Other authors have previously speculated on the presence of a delta-lactonase in the liver (4). Our data provide evidence for the presence of a heat-labile enzyme in the serum which is capable of rapidly converting the

mevalonolactone to the corresponding salt under both in vivo and in vitro conditions. This set of observations demonstrates, for the first time, the presence of a delta-lactonase (possibly mevalonolactone hydrolase) in rat serum.[1] Studies are now under way to characterize and isolate this enzyme.

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[1] Recently, Dr. Tom Parker informed us (personal communication) that he has found a heat-labile, non-dialyzable enzyme within the liver that hydrolyzes the mevalonolactone to the salt form. Since completion of our study, Dr. Parker has presented his studies on this hepatic hydrolase (10).