RHYTHMIC ACTIVITY OF UTILIZATION OF MEVALONATE FOR BIOGENESIS OF CHOLESTEROL

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SUMMARY

The utilization of mevalonate for biogenesis of cholesterol shows rhythmic activity with a peak at midnight and the step responsible is likely to be between mevalonate and isopentenyl pyrophosphate.

It is now well-established that biogenesis of cholesterol in the liver is subject to rhythmic changes during a day with a peak at midnight. This had been demonstrated by a number of experiments in several laboratories around the world. These include: 1. incorporation of labelled acetate into cholesterol by slices of liver *in vitro* (1); 2. incorporation of labelled acetate into hepatic cholesterol (2,3) *in vivo*; and 3. activity of hepatic microsomal enzyme, 3-hydroxy-3-methyl glutaryl CoA (HMG CoA) reductase (EC. 1.1.1.34), the "rate-limiting" enzyme in isoprene pathway, responsible for the synthesis of mevalonate (4-6). It was, however, found that the activity of HMG CoA reductase increased 10-fold at midnight compared to noon, whereas that of incorporation of acetate into cholesterol, both *in vitro* and *in vivo*, increased only 3-fold. Subba Rao & Ramasarma (2) hypothesized that there may be another step in the pathway, besides HMG CoA reductase, that becomes rate-limiting at night. We now report that utilization of mevalonate also shows rhythmic activity with a peak at midnight and the step res-

ponsible is likely to be between mevalonate and isopentenyl pyrophosphate.

MATERIALS AND METHODS

Male albino rats weighing 110-130g obtained from the Institut colony were housed in a room with a light period from about 6.00-18.00 h. They were given Hindustan Lever rat pellet diet and water ad libitum. Groups of rats were killed at times indicated and the livers removed and chilled in ice-cold 0.25M sucrose solution. Homogenates of the livers were made in freshly prepared 0.25M sucros (9 ml/g tissue) solution and were centrifuged at 800xg for 10 min to obtain post-nuclear supernatants. The incubation mixture for the incorporation of [2-^{14}C]mevalonate into the sterol fraction consiste of the following: potassium phosphate buffer, pH 7.4 (250 µmol), ATP (5 µmol), $MgCl_2$ (20 µmol), nicotinamide (6o µmol), [2-^{14}C]RS-mevalonate (1.52 x 10^5 c.p.m., 300 nmol) and post-nuclear supernatan (1 ml), in a total volume of 2.0 ml. The incubation was carried out for 1 h at 37°C in a metabolic shaker, and the reaction was stopped by adding 20 ml of 80% aq.ethanol. The contents were saponified and processed for nonsaponifiable lipids, sterol fraction was separated and radioactivity measured as described by Ranganathan & Ramasarma (and Joshi et al. (8). The specific activity is expressed as nmol of mevalonate converted per h per mg of protein. The reaction mixture for the release of $^{14}CO_2$ from [1-^{14}C]mevalonate was essentially the same as above except that nicotinamide was omitted and [1-^{14}C]RS-mevalonate (1.25 x 10^5 c.p.m., 300 nmol) was used as the tracer in a total volume of 1.7 ml. The incubation was carried out in stopper Warburg flasks at 37°C without shaking. The center well contained 0.2 ml of 1M KOH to absorb the released CO_2. After an incubation of 20 min 0.5 ml of 1N H_2SO_4, contained in the side arm, was tipped in to stop the reaction and the flasks were left at room temperature for 1 h to complete the CO_2 absorption. Aliquots of the center well contents were taken for measuring the radioactivity (7). The specif activity is expressed as nmol of mevalonate converted per h per mg o protein. The concentration of mevalonate used in both cases was at saturation level so that rates measured correspond to the overall activity of the enzyme systems under the incubation conditions. The activities are measured immediately after killing at different time periods of the 24 h day as stated.

RESULTS AND DISCUSSION

In the first set of experiments, [2-^{14}C]mevalonate was used to estimate the rate of biogenesis of sterols from mevalonate. It can be seen from the data in Fig. 1 that an increase in the activity was observed during the night hours with a peak at midnight. Compared to noon values, the values obtained between 18.00-08.00 h were all significant. In the second set of experiments, [1-^{14}C]-mevalonate was used and the amount labelled CO_2 liberated was measured. This system would measure the overall rate of three

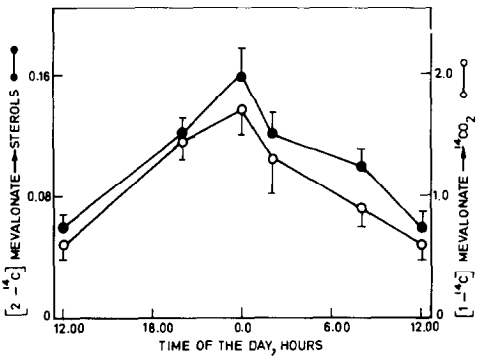

Fig. 1: Rhythmic activity of utilization of mevalonate. Incorporation of [2-^{14}C]-mevalonate into sterols and release of $^{14}CO_2$ from [1-^{14}C]mevalonate were tested by using homogenates of liver from rats killed at different times of 24 h day as described in the text. The activities are given as nmol/h/mg of protein (mean with one S.E.M.) with 8 animals for 0.00, 12.00 and 20.00 hours, and 4 animals for 2.00 and 8.00 hours in each group. Compared with values at 12.00 hours all the increases are significant ($P<0.01$).

enzymes viz. mevalonate kinase, mevalonate-5-phosphate kinase and mevalonate-5-pyrophosphate decarboxylase. All the three steps use a molecule each of ATP, and CO_2 is liberated in the last one yielding isopentenyl pyrophosphate as the product. Similar rhythmic activity was also observed with this system with a peak at midnight. These results suggest that besides HMG CoA reductase, there must be another enzyme in the isoprene pathway between mevalonate and isopentenyl pyrophosphate, which exhibits rhythmic changes in activity. Since the change between midnight to noon values in this segment of pathway was about 3-fold, it appears likely that the overall rate will be determined by this step at midnight. In this connection, it is appropriate to mention that Back *et al.* (1) had tested in a similar system *in vitro* and found that percent incorporation of [2-^{14}C]-mevalonate had remained unchanged in animals tested between 12.00-

14.45 h and 23.40-01.00 h. However, in these experiments they had used 50 times higher concentration of mevalonate than required for saturation of the substrate and therefore it is uncertain whether they measured the actual rates as done in our experiments. This wil be the first report of rhythmic activity of utilization of mevalonat in the isoprene pathway.

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