Regulation of 5-methyltetrahydrofolate synthesis

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After an intraperitoneal injection of 100 μ mol of methionine to rats, there is rapid oxidation of the methyl group of hepatic 5-methyltetrahydrofolate to formate and CO₂. Recovery of the methylfolate level starts 2.5 h after the methionine injection, when the hepatic methionine level and the *S*-adenosylmethionine/*S*-adenosylhomocysteine ratio have returned to baseline values. *S*-Adenosylmethionine concentration is still elevated at this time.

INTRODUCTION

The enzyme 5,10-methylenetetrahydrofolate (5,10-CH₂H₄folate) reductase (EC 1.5.1.20) is a dimeric flavoprotein converting 5,10-CH₂H₄folate to 5-methyltetrahydrofolate (5-MeH₄folate). The thermodynamics of the reaction *in vitro* are strongly in favour of Mefolate synthesis and the reverse reaction, on the basis of studies *in vitro*, is unlikely to occur (Green *et al.*, 1988). *In vitro* the enzyme is inhibited by *S*-adenosylmethionine (AdoMet) (Kutzbach & Stokstad, 1971), but not by methionine.

In vivo, however, Noronha & Silverman (1962) showed that methionine added to rat diet caused a loss of MeH₄folate from liver, with a corresponding increase in formylH₄folate and unsubstituted H₄folate. This has been confirmed most recently by Brody *et al.* (1982) and Lumb *et al.* (1988). Lumb *et al.* (1988) found that methionine was far more active in this respect than AdoMet. MeH₄folate has been found to be an active methylating agent for biogenic amines, but is first oxidized to CH₂H₄folate by methylene reductase before transfer of a one-carbon unit (Pearson & Turner, 1975; Taylor & Hanna, 1975). There is thus a difference between the findings *in vivo* and *in vitro* on whether methylene reductase is capable of oxidizing MeH₄folate. This study was planned to provide further data on this matter.

MATERIALS AND METHODS

Approx. 100 g-body-weight Sprague–Dawley rats were given an intraperitoneal injection of 100 μ mol of methionine and killed at half-hourly intervals thereafter up to 4 h, livers were removed immediately and a portion heated for 5 min in 20 vol. of 1% (w/v) ascorbic acid, pH 7, at 95 °C. Samples were then cooled, homogenized, and stored at –20 °C. A further portion of liver was deproteinized with 10 vol. of methanol for methionine assay, and a third aliquot was deproteinized with 10 vol. of HClO₄ for AdoMet and *S*-adenosylhomocysteine (AdoHcy) assay.

In some studies folate analogues were separated by DEAE-cellulose chromatography after conversion into folate monoglutamate and folates, which were identified both by their elution position and microbiological assay with *Lactobacillus casei* and *Pediococcus cerevisiae*

(Lumb *et al.*, 1988). Latterly, liver homogenates were assayed directly with *L. casei* and *Streptococcus faecalis* without prior chromatography, and MeH₄folate levels were obtained by subtracting the *Strep. faecalis* value (which measures folates other than MeH₄folate) from the *L. casei* value (which measures total folates). Methionine was measured by h.p.l.c. as described by Bottiglieri (1988).

AdoMet and AdoHcy were separated on an isocratic system with a reverse-phase column (25 cm $\times$ 5 cm; 5 μ ODS Hypersil) eluted with 0.1 M-sodium acetate, pH 4.40, containing heptanesulphonic acid (5 mM) and 4.4% (v/v) acetonitrile at a flow rate of 1.0 ml/min. Detection was carried out with u.v. light at 254 nm. The AdoMet standard was generously supplied by Dr. G. Stramentonoli of BioResearch Laboratories, Liscate, Milan, Italy. The AdoHcy standard was obtained from Sigma Chemical Co., Poole, Dorset, U.K.

RESULTS

These are shown in Fig. 1. After methionine injection, the liver MeH₄folate fell rapidly from 4.6 nmol/g of liver to 0.7 nmol/g. There was a significant rise in the MeH₄folate level at 2.5 h, and preinjection levels were regained at 4 h.

There was a marked increase in liver methionine, AdoMet and AdoHcy levels. The methionine level returned to its baseline value at 2 h, and the AdoMet level at 4 h, but the AdoHcy level was still elevated at 4 h. The AdoMet/AdoHcy ratio was normal at 2.5 h.

DISCUSSION

There are two interpretations possible about the factors influencing a resumption of MeH₄folate synthesis. The simplest is that the reaction is sensitive to the end product of methylfolate metabolism, which is methionine.

The second is that resumption of MeH₄folate synthesis occurs after a return of the AdoMet/AdoHcy ratio to normal. *In vitro*, AdoMet inhibits methylene reductase activity. In the present study, however, the Mefolate level rose despite the AdoMet level being raised. AdoHcy is an inhibitor of transmethylation reactions by AdoMet, presumably by competing with AdoMet for its enzyme-

Abbreviations used: 5,10-CH₂H₄folate, 5,10-methylenetetrahydrofolate; 5-MeH₄folate, 5-methyltetrahydrofolate; AdoMet, *S*-adenosylmethionine; AdoHcy, adenosylhomocysteine.

Fig. 1. Effect of methionine on folate and related compounds

A 100 μ mol portion of methionine was given intraperitoneally to rats. Four rats were killed before the injection and four at 30 min intervals. Livers were removed and the hepatic levels of MeH₄folate, methionine, AdoMet, AdoHcy and the AdoMet/AdoHcy ratio were measured.

binding site. It is possible that, *in vivo*, AdoHcy may compete with AdoMet for an allosteric site on methylene reductase, as shown by Kutzbach & Stokstad (1971) with partially purified enzyme. At elevated AdoMet levels, AdoMet inhibition of the enzyme prevails, but when AdoMet has declined after 2 h and the AdoMet/AdoHcy ratio has normalized, there is relatively more AdoHcy on the receptor. Assuming that AdoHcy is not an inhibitor of methylene reductase, the decreased AdoMet binding to the receptor may allow a return of methylene reductase activity.

On the other hand, *in vivo*, methionine is much more active than an equimolar amount of AdoMet in causing Mefolate oxidation (Lumb *et al.*, 1988), although this assumes that both are taken up by cells in equal amounts. In rats in which cobalamin has been inactivated by nitrous oxide, AdoMet is totally inactive in initiating oxidation of MeH₄folate, but methionine retains its activity (M. Lumb, R. Deacon, J. Perry & I. Chanarin, unpublished work). The balance of evidence is therefore that data obtained *in vitro* do not reflect the physiological situation and that the methionine level is the main factor in controlling hepatic levels of MeH₄folate.

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