Regulation of malic enzyme expression in hepatocytes in culture

DAVID MANN,* WALTER BARTLEY,* ALISTAIR J. STRAIN† and ERNEST BAILEY*
*Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, U.K., and †School of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

Previous studies in vivo have demonstrated that in neonatal and adult rats hepatic malic enzyme expression is controlled at the pretranslational level by dietary and hormonal factors [1, 2]. However, in order to demonstrate that particular effectors have a direct effect on gene expression in liver, techniques in vitro, such as the use of primary cultures of non-proliferating rat hepatocytes, have to be adopted. Unfortunately, the widely used practice of plating cells on type 1 collagen leads to decreased hepatocyte function as evidenced for example by diminished albumin production [3]. Recently, a new substratum, EHS matrix, has been employed which appears to result in greater maintenance of liver specific gene expression [4, 5]. In the present study, the response of malic enzyme gene expression to various putative effectors has been compared in adult hepatocytes plated on to type 1 collagen or Engelbreth–Holm–Swarm (EHS) matrix. Malic enzyme activity has been determined in cell lysate supernatant solutions and dot–blot and Northern blot analysis has been used to determine the amount and size of malic enzyme and albumin mRNA in hepatocytes maintained in culture for either 4 or 6 days in serum-free chemically defined medium containing 5 mM-glucose.

Attachment of isolated hepatocytes was similar on collagen and EHS matrix, with approx. 80% of cells attached after 60 min. Hepatocytes plated on collagen flattened within 24 h to form confluent monolayers of polygonal, epithelial-like cells on EHS matrix whereas those on EHS matrix cells remained spherical within 24 h to form three-dimensional clusters of 10–20 cells as previously reported [5]. The levels of all parameters measured (malic enzyme mRNA and activity, albumin mRNA and lactate dehydrogenase activity) were very similar in cells assayed immediately after attachment to either substratum to those detected in the whole animal. Such post-plating values were standardized with those of the normal adult rat liver to facilitate a comparison of the magnitude of the responses of malic enzyme expression to the various effectors in vivo and in vitro.

Hepatocytes plated on collagen and maintained in culture for 4 days exhibited little response to tri-iodothyronine, insulin or dichloroacetate with regard to malic enzyme activity or mRNA levels. In addition, the amounts of albumin mRNA were markedly reduced compared with the levels determined in cells immediately after isolation. However, after 6 days in culture the effectors did cause increases in enzyme activity and mRNA levels relative to control cells which were found to have markedly reduced levels of malic enzyme expression compared with freshly plated cells. The levels of malic enzyme induction observed were considerably less than those arising in vivo after treatment with the appropriate effector.

Dramatic differences were observed when hepatocytes were incubated on EHS-coated culture dishes. The level of malic enzyme induction after 4 days of treatment with the appropriate effector corresponded to that recorded after 6 days’ stimulation of cells on the collagenous substratum. After 6 days in culture using EHS matrix, increased induction of malic enzyme activity and mRNA was observed with tri-iodothyronine and dichloroacetate such that a similar response was obtained to that recorded in vivo. In addition, EHS-maintained cells produced albumin mRNA at about twice the level found in cells immediately after isolation, thus indicating maintenance of hepatic function.

In order to try to elucidate the nature of potential metabolic intermediates involved in the induction of malic enzyme expression, hepatocytes plated on EHS gel were maintained for 6 days in culture medium containing 5 mM-glucose and either malate (5 mM), fructose (5 mM) or high glucose concentrations (25 mM, final). Malate caused induction of malic enzyme in the absence of insulin, whereas fructose and high glucose concentrations only caused increased enzyme expression in the presence of insulin. The direct or indirect role of malate in malic enzyme induction merits further investigation.

Thus use of EHS matrix provides a cell-culture system for hepatocytes which resembles the situation in vivo for enzyme induction. However, a note of caution is necessary, since Northern blot analysis demonstrates that whereas in vivo and in freshly isolated cells the ratio of 2.0 kb to 3.1 kb malic enzyme mRNA species was 0.5–0.8, with 4 days in culture. Paradoxically, hepatocytes cultured on EHS matrix may provide an excellent system in which to study the factors involved in the production of the various malic enzyme mRNA species.


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Regulation of hepatic malic enzyme mRNAs during development

DAVID MANN, WALTER BARTLEY and ERNEST BAILEY
Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, U.K.

Much of the information on the developmental, hormonal and nutritional regulation of rat hepatic malic enzyme has been derived from studies of enzyme activity and immuno-reactive protein [1], thus limiting information to the post-translational level. The isolation of a cDNA (pME6) for rat liver malic enzyme [2] has permitted studies of a pre-translational regulation of expression of the enzyme in adult rats [3]. In the present study the use of the cDNA (pME6) in dot–blot and Northern blot analysis has facilitated an investigation of developmental changes in total rat hepatic malic enzyme mRNA levels and also in three malic enzyme mRNA species.
and the effect of dietary and hormonal influences at the suckling/weaning transition.

Dot–blot analysis of malic enzyme mRNA was carried out with both poly(A)+ RNA isolated from total liver RNA and liver polysomal poly(A)+ RNA from rats at various stages of development from late fetal to adult life. Small amounts of malic enzyme mRNA were detected in RNA isolated from fetal rats of 21 days gestation. After birth, malic enzyme mRNA levels decrease five-fold to the very low level which is present until 16 days of age, after which time it increases rapidly both before and after weaning (at 21 days of age) to reach a peak at 35 days of age, thereafter falling to adult values. These postnatal changes in amounts of malic enzyme mRNA parallel the changes reported for enzyme activity and immunoreactive protein [1], indicating pre-translational control of enzyme expression during development of the rat liver. In contrast to malic enzyme, levels of albumin mRNA increase slightly during the neonatal period but attain a stable value by about the third week of life, as has previously been reported [2].

In order to determine the size of the malic enzyme mRNA present at different stages of hepatic development, Northern blot analysis was performed. Such analysis demonstrated the presence of three malic enzyme mRNA species in late fetal liver with sizes of 1.6 kb (60% of total), 2.0 kb and 3.1 kb. In contrast, only the 2.0 kb and 3.1 kb species of mRNA were observed in normal postnatal rat liver with the relative proportions varying during development. Thus at 16–18 days of age the larger form predominates. However, although levels of both species increase after weaning a relatively greater increase in the amount of the 2.0 kb species results in this mRNA being four times more abundant than the 3.1 kb species at 35 days of age. As the level of total malic enzyme mRNA falls to adult values the ratio of 2.0 kb to 3.1 kb species falls to 3, as previously reported for adult rat liver [2].

Similar analysis of hepatic albumin mRNA at various stages of development revealed only one species of mRNA, 2.2 kb in size, in agreement with previous observations [4].

Experiments have been carried out to characterize the agents responsible for the large increase in malic enzyme expression at weaning. Both tri-iodothyronine and dichloroacetate, alone or in combination, have been shown to induce malic enzyme activity and immunoreactive protein in neonatal rats [1]. The present study demonstrates that such changes are secondary to an elevation of the malic enzyme mRNA species and that malic enzyme expression is only slowly responsive to the effectors used. Northern blot analysis revealed that the 2.0 kb and 3.1 kb malic enzyme mRNA species were induced to the same extent and that the 1.6 kb mRNA species is transiently expressed at short time intervals after treatment of 10-day-old animals with tri-iodothyronine and dichloroacetate.

In order to investigate the effects of dietary change at the suckling/weaning transition on the developmental expression of hepatic malic enzyme, rats were weaned at 21 days of age on to either the stock laboratory diet (high starch), a high-fat diet or a high-sucrose diet. Relative to the stock-diet-fed animals, weaning on to the high-sucrose diet resulted in increased malic enzyme activity, whereas weaning on to the high-fat diet resulted in reduced malic enzyme activity. Changes in enzyme activity were accompanied by concomitant changes in malic enzyme mRNA levels. Northern blot analysis revealed no significant differences in malic enzyme mRNA species between the various experimental groups. Premature weaning of animals at 16 days of age on to the stock laboratory diet resulted in premature induction of malic enzyme activity, mRNA production and a change in proportions of the 2.0 kb and 3.1 kb mRNA species.

The present study indicates that developmental changes in rat hepatic malic enzyme expression are controlled at the pre-translational level. Further, developmental changes in various malic enzyme mRNA species occur, the control of and physiological significance of which remain to be elucidated.


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Tissue uptake of lithium in guinea-pig isolated intestinal mucosa after chronic lithium ingestion

JONATHAN D. PHILLIPS, ROBERT J. DAVIE and NICHOLAS J. BIRCH

Biomedical Research Laboratory, School of Health Sciences, Wolverhampton Polytechnic, Lichfield Street, Wolverhampton WV1 IDI, U.K.

Lithium is effective in the prophylaxis of recurrent affective disorder, yet its mode of action is not fully understood [1]. It has been shown that there is often a delay of some weeks before the clinical benefits of lithium treatment become fully apparent [2]. This delay has been linked to gradual inhibition of lithium-sodium countertransport across cell membranes [3]. Lithium is administered orally, and previous experiments in vivo showed that tissue lithium uptake of lithium into the cells of the absorptive intestinal mucosa in lithium-naive guinea pigs is negligible [4, 5]. Further experiments demonstrated that tissue uptake of lithium into the cells of the absorptive intestinal mucosa in lithium-naive guinea pigs is negligible [6]. The experiments described here were performed to investigate the effects on tissue lithium uptake of chronic administration of lithium to guinea pigs in vivo.

Dunkin–Hartley guinea-pigs (n = 6) were treated with lithium chloride (25 mmol/l) in the drinking water for at least 21 days before the study. Terminal anaesthesia was administered as follows: premedication with atropine sulphate (0.06 mg) and sodium pentobarbitone (30 mg/kg total body weight), followed by a neuroleptic analgesic combination (5 mg of droperidol/kg, and 1 mg of phenoxydone/kg) [7]. When the animals were completely anaesthetized, laparotomy was performed and the small intestine was located. Small pieces of intestine were removed serially, opened longitudinally, and the absorptive mucosal epithelium was stripped from the underlying muscle and connective tissue layers to produce isolated intestinal mucosa [8]. Initial tissue lithium concentrations were estimated in samples of isolated intestinal mucosa after digestion using NCS tissue solubilizer (Amersham International, Amersham, Bucks, U.K.). Further, samples of isolated mucosa were mounted in small Perspex chambers [8] for tissue lithium uptake studies over 45 min at