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ScienceDirect
Life Sciences 79 (2006) 1764–1772
Life Sciences
www.elsevier.com/locate/lifescie

Serum ethanolamine and hepatocyte proliferation in perinatal and partially hepatectomized rats

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Received 4 March 2006; accepted 8 June 2006

Abstract

It has been shown that the administration of ethanolamine (Etn) to partially hepatectomized rats enhances stimulation of DNA synthesis in regenerating hepatocytes. The present study aimed to test the hypothesis that the level of serum Etn *in vivo* may be regulated to control the growth of hepatocytes. Concentrations of serum Etn were determined in rats 1) of varying ages (from embryonic-19 (E-19) to 7-week-old), and 2) during regeneration following two-thirds hepatectomy (PH), to investigate whether serum Etn concentration correlates with the rate of proliferation of hepatocytes in growing animals or during regeneration. Serum Etn levels were 3 fold higher in E-19 fetuses and newborns than in adults, and were increased 2 fold 4 h after PH and remained high for at least 24 h. Results in both systems indicated a significant positive correlation between the rate of hepatocyte proliferation and serum Etn levels. Furthermore, Etn supplementation of 0.1 to 1 mmol immediately after PH promoted a significant weight gain and stimulated phosphatidylethanolamine (PE) and phosphatidylcholine (PC) synthesis in the regenerating liver. We also observed that whenever serum Etn levels were elevated, the metabolism of PE and PC in the liver changed dynamically, first by elevating the net synthesis of PE. Taken together, these results suggested that the levels of serum Etn might be regulated based on the physiological state of an animal, which consequently regulates the proliferation of hepatocytes.

Keywords: Ethanolamine in serum; Phosphatidylethanolamine; Membrane phospholipids; Hepatocyte proliferation; Liver regeneration

Introduction

Mammalian epithelial cells including primary hepatocytes (Ajioka et al., 2002; Kume and Sasaki, 2006; Nelson et al., 1996; Sasaki et al., 1997, 1998) require ethanolamine (Etn) to proliferate *in vitro*, and stimulation of growth by Etn occurs in a dose-dependent manner (Babcock et al., 1983; Hammond et al., 1984; Kano-Sueoka et al., 1982, 2001; Lechner et al., 1982; Peehl and Stamey, 1986; Tsao et al., 1982). Epithelial cells require Etn, because without it, they are unable to synthesize amounts of phosphatidylethanolamine (PE) necessary for sustaining growth (Kano-Sueoka and King, 1988). Without Etn supply, PE content in cell membranes is halved, whereas that of phosphatidylcholine (PC) is increased by 30% (Kano-Sueoka and King, 1988; Kano-Sueoka et al., 1990), and cellular functions associated with cell membranes become abnormal and growth stops (Fisk and Kano-Sueoka, 1992; Kano-Sueoka et al., 1983; Kano-Sueoka and King, 1987; Kano-Sueoka and Nicks, 1993).

In contrast to the *in vitro* situation, the importance of Etn *in vivo* remains unclear. We previously found that administration of Etn to rats enhanced stimulation of DNA synthesis in hepatocytes after partial hepatectomy (Sasaki et al., 1997). This finding suggested that Etn might also be an important factor *in vivo* for the proliferation of epithelial cells including hepatocytes that were Etn-dependent for growth *in vitro*. We hypothesize that serum Etn plays a role in regulating growth of hepatocytes, and perhaps other epithelial cell types *in vivo*. To test this hypothesis, we examined whether serum Etn concentration became elevated when there was a large number of proliferating hepatocytes. We also investigated whether administration of Etn promoted proliferation of hepatocytes *in vivo* in the regenerating liver.

Little is known about how stable or variable serum concentrations of Etn are. Moreover, nothing is known about how or to

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0024-3205/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.lfs.2006.06.036

what extent serum Etn is regulated. A limited amount of available data suggests that the concentration of serum Etn may be variable. Dickinson et al. (1965, 1970) analyzed concentrations of free amino acids, including that of Etn, in blood plasma in human adults and newborn infants. Their results showed that the average concentration of Etn in adult plasma was 1.6 μM (traces up to 11 μM), whereas in newborn infants it was 52.5 μM (26–92 μM). In contrast, levels of free amino acids in adults and newborns were relatively similar. Houweling et al. (1992) found that serum Etn levels increased from 29 to 50 μM during the first day after two-thirds hepatectomy (PH) in rats. These findings suggested that serum Etn levels can be variable and may be regulated according to demand by cells requiring Etn to grow. Thus, in newborn infants or during liver regeneration, the population of growing Etn-requiring cells would be significantly larger than those in adults or in animals with an intact liver. If the above assumption is correct, it is also possible that supplementation with Etn may further promote the growth of Etn-requiring cells.

In the present study, we used two rat model systems; one consisted of varying ages from E-19 to 7-week-old, and the other model consisted of varying stages of regenerating liver. In both systems, we determined concentrations of serum Etn, rates of hepatocyte proliferation [data for hepatocyte proliferation during regeneration have already been documented (Bucher and Swaffield, 1964; Lieberman and Kane, 1965)] and levels of hepatocyte phospholipids.

Rat and human milk contains high levels of Etn and phosphoethanolamine (PEtn), which can be used for PE synthesis (Jensen, 1995; Kanno et al., 1997). However, it is not known whether their content in milk changes with time after parturition. Therefore, we analyzed the milk of nursing mothers for two weeks after parturition, and compared data with those of serum Etn levels, and with the rate of hepatocyte proliferation in perinatal animals. Our assumption was that milk obtained immediately after parturition would contain higher amounts of Etn and PEtn to meet the demands of the newborn.

Although the process of regeneration of a partially hepatectomized (PHed) liver has been well studied, phospholipid metabolism in the regenerating liver is not fully understood. With regards to PE, Houweling et al. (1992) noted that one day after surgery, concentrations of serum Etn were increased by about 70%, and the rate of PE synthesis was also increased. In the present study, we sought to determine a detailed time course of the changes in the amount of serum Etn and of phospholipids found in the regenerating liver. Were then analyzed results in relation to the way hepatocytes proliferated after PH.

From the results we sought to show that when the rate of hepatocyte proliferation was high, serum Etn levels were also high both in growing animals and in the regenerating liver. In addition, phospholipid profiles in the liver were seen to dynamically change relative to serum Etn levels and the rate of hepatocyte proliferation.

Experiments were carried out to determine whether the administration of Etn promoted the growth of hepatocytes and liver regeneration. We showed that Etn supplementation hastened the recovery process, most likely by stimulating PE and PC synthesis.

We experimentally demonstrate our hypothesis that serum Etn concentrations are regulated in response to demands made by the epithelial cell population. In growing epithelial cells, a sufficient supply of Etn is shown to be necessary to synthesize PE in order to achieve a suitable membrane phospholipid environment for cellular signaling.

Materials and methods

Animals and experimental design

Sprague–Dawley rats were purchased from Charles River, Japan, and maintained on standard chow (CRF-1, from Oriental Yeast Co., LTD, Japan). Rats were handled according to protocols approved by the Food Science Institute of Meiji Milk Dairies Corporation, which follow the Guide for the Care and Use of Laboratory Animals (NRC1996). Animals bred in the laboratory were used to examine effects of age on serum Etn concentrations and liver phospholipids. Anesthetized animals, E-19, 0, 2-, 7-, 14-, 28-, and 49-day-old rats, were sacrificed using diethylether after withdrawing blood and removing the liver. Animals older than 4 days were identified by sex, and males and females were analyzed separately. There were no significant differences in serum levels of Etn and amino acids by sex.

Pregnant rats that carried E-19 or day(d)-20 fetuses were used to examine how Etn (50 mg in hydrochloride salt, Sigma Chemical Co, St. Louis, MO) given by peritoneal injection was transported from mother to fetus. Two, 4, and 6 h after Etn administration, blood was withdrawn from the main artery of the pregnant mother and from the heart of the fetuses. Blood was also withdrawn from the tail vein, placenta, and umbilical cord of the pregnant rat. Rats that had nursed for 2 to 14 days after parturition were used for analysis of concentrations of Etn and PEtn in milk.

Two-thirds hepatectomy PH was carried out as previously described using 7-week-old male rats (Sasaki et al., 1997). To investigate the effects of Etn supplementation, the hydrochloride salt of Etn dissolved in saline (0.1 mmol or 1 mmol Etn/ml rat) or saline was intraperitoneally injected into rats within a minute after PH. Injection of the same dose of Etn or saline was repeated every 24 h. Anesthetized animals were sacrificed using diethylether after withdrawing blood from the main artery and removing the liver at 0, 2, 4, 8 and 24 h after PH for serum Etn and liver phospholipids Animals were also sacrificed at 1, 2, 3, and 7 days after PH for liver weight.

Blood collection and amino acid analysis

Mother rats carrying E-19 fetuses were anesthetized with pentobarbital solution at 50 mg/kg body weight, and blood of E-19, and 0, 2, and 7-day-old rats was withdrawn from the heart. In some cases, blood was collected from the main artery and from the heart of the same animal to compare levels of Etn and amino acids. We found that Etn and amino acid levels were the same from both sources, respectively. Blood was allowed to clot at 4 °C, and serum was collected and stored at −20 °C until ready for analysis. For analysis of E-19 fetuses, blood samples were pooled from two fetuses to allow for duplicate analysis.