ON THE POSSIBILITY THAT THE PREREPLICATIVE INCREASES IN ORNITHINE DECARBOXYLASE ACTIVITY ARE RELATED TO DNA SYNTHESIS IN LIVER

Dennis J. GAZA, John SHORT and Irving LIEBERMAN

Department of Anatomy and Cell Biology, University of Pittsburgh School of Medicine, and Veterans Administration Hospital, Pittsburgh, Pa. 15261, USA

Received 26 March 1973

1. Introduction

Removal of part of the liver of the rat is not followed immediately by an increase in DNA replication in the remnant cells. Rather there is a prereplicative period of about 14 hr. It is reasonable to expect that critical changes must take place in the liver during this time in order for the nuclei to enter the S period. The critical changes may culminate in the synthesis of proteins that are needed for the replicative process.

Alterations in RNA and protein synthesis appear to be obligatory events of the prereplicative period after partial hepatectomy [1, 2]. No other liver change is known to be requisite to DNA formation. The dearth of information is not due to a failure to find alterations in regenerating liver. On the contrary, it is a reflection of the large number of changes that have been described and of the lack of a suitable means for selecting for intensive study those changes that show promise of being related to the later synthesis of DNA.

The availability of two new procedures that induce hepatic DNA synthesis in unoperated rats now offers a means for at least tentatively assigning a causal relationship between a prereplicative change and the replication of DNA. DNA formation can be induced in the liver of the intact animal with a hormone-containing solution (TAGH solution) [3] and by shifting rats from a protein-free to a protein-containing diet [4]. Any prereplicative change that takes place after partial hepatectomy but fails to occur after both of the other stimuli can be excluded as being essential for DNA synthesis. The appearance of the same change in all three systems, on the other hand, is consistent with a relationship between the change and the induction of DNA replication.

Russell and Snyder [5] and Jänne and Raina [6] have described a large increase in the activity of liver ornithine decarboxylase after partial hepatectomy of the rat. The observations of Schrock et al. [7] cast some some doubt on a role for this change in the formation of DNA. They found that agents that do not stimulate liver growth (hypertonic glucose given intravenously; Celite given intraperitoneally) also cause a large increase in the decarboxylase activity. Unlike the prolonged increase in the activity after partial hepatectomy, however, even continued infusion of hypertonic glucose produced only a brief rise in the decarboxylase activity with a peak at 2 hr after the start of treatment.

More recently, in a careful time study, Höltä and Jänne [8] showed that the increase in ornithine decarboxylase activity after 70% hepatectomy is biphasic, one peak being at 4 hr, the second, at about 11 hr, after the operation (140–160 g rats). The possibility that at least the second rise in the enzyme activity has some relationship to the later formation of DNA has led us to measure the decarboxylase in unoperated rats as a function of time after injection of the TAGH solution and after the shift from a protein-free to a protein-containing diet.

2. Materials and methods

Female rats, Fischer 344, were used when they weighed about 120 g. Partial hepatectomy refers to the removal of 70% of the liver (remaining were the right lateral, caudate and accessory lobes). The TAGH solution (7 ml) (triiodothyronine, 100 μg; amino acids, 150 mg; glucagon, 1 mg; and heparin, 100 U.S.P. units)

was injected subcutaneously. The protein-free diet contained cellophane spangles, 12%; glucose, 12%; corn starch, 60%; "Vitamin Fortification Mix", 2%; "Salt Mix, U.S.P. XIII No. 2", 4%; and corn oil, 10%, and was obtained from General Biochemicals, Chagrin Falls, Ohio, USA. Supplementation with 40% casein was at the expense of the corn starch. The preparation of the cytosol fraction of liver and the estimation of ornithine decarboxylase were exactly as described by Jänne and Williams-Ashman [9].

3. Results

In confirmation of the data of Höltta and Jänne [8], 70% hepatectomy caused biphasic increases in the activity of liver ornithine decarboxylase (fig. 1A). The first peak of activity was at 4 hr after the operation, the second, at 9 to 11 hr. Fig. 1B shows that similar results were obtained after the injection of the TAGH solution into unoperated animals except that the biphasic nature of the response was even more pronounced than after partial hepatectomy. The figure does not show that injection of 0.15 M NaCl caused no increase in the enzyme activity. Finally, two periods of increased enzyme activity followed the shift of protein-deficient rats to a protein-containing diet (fig. 1C). In this case, the peaks of activity were smaller than after partial hepatectomy or treatment with the TAGH solution.

The enhancement of liver ornithine decarboxylase activity after 70% hepatectomy is not dependent upon the adrenal glands [10]. The TAGH solution raised the activity of the enzyme in rats from which the adrenal or pituitary glands had been extirpated. Thus, measured at 11 hr after the injection, the specific activities of the enzyme in intact, adrenalectomized and hypophysectomized animals were 1.1, 1.2 and 0.7 nmoles/mg protein/hr, respectively.

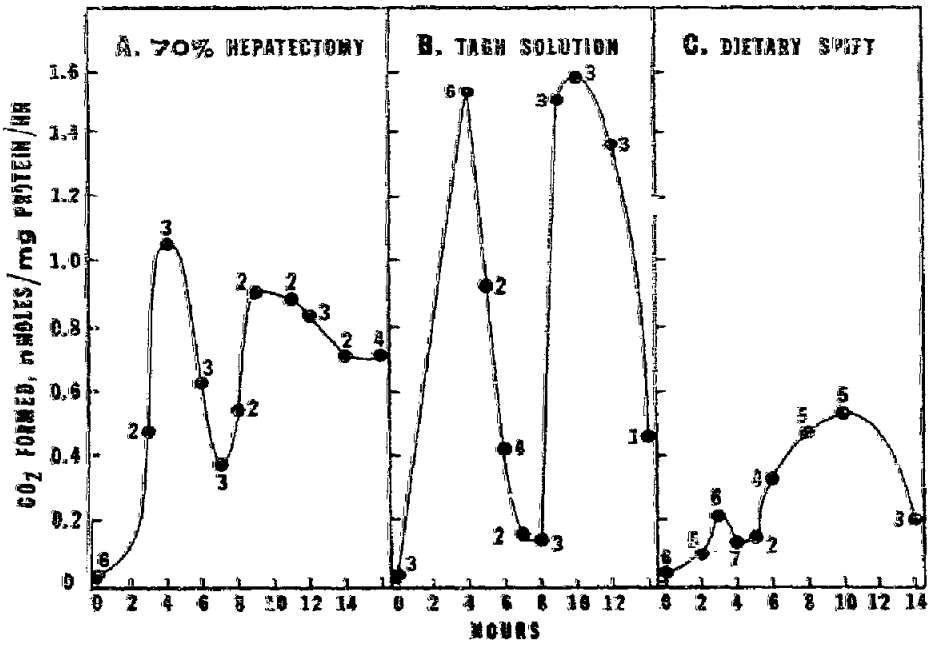

Fig. 1. Liver ornithine decarboxylase activity as a function of time after 70% hepatectomy, injection of intact rats with the TAGH solution and a shift of unoperated animals from a protein-free to a 40% protein diet. In all cases, liver samples were taken between 6 AM and 10 AM. For C, rats that had been freely fed pellets of Purina Laboratory Chow (24% protein) were given a protein-free mash for 3 days. At the end of this time (8 AM), food was removed. The animals were presented with a mash containing 40% casein at zero time. The specific activity of liver ornithine decarboxylase in animals that were maintained on the protein-free diet for 3 days was the same at 6 PM, 2 AM and 6 AM. Each point represents the average of the individual results with 1 to 7 rats as shown.

4. Discussion

The TAGH solution has already been used to gain some insight into the pertinence to DNA synthesis of two of the prereplicative liver changes that take place after 70% hepatectomy. The marked rise in the plasma membrane activity, alkaline phosphatase, that follows the operation was absent in intact rats that had been given the the TAGH solution despite the fact that the stimulation of DNA synthesis was almost as great as after partial hepatectomy [11]. On the contrary, almost identical biphasic increases in cyclic AMP were found in liver after 70% hepatectomy and treatment of intact rats with the hormone solution [12], suggesting that the nucleotide plays a role in the regulation of hepatic DNA replication.

The present results provide some support for a connection between the rises in ornithine decarboxylase activity and the ability of the liver nucleus to replicate its DNA. It cannot be assumed, of course, that the connection resides in the enzyme activity itself. Instead, it may be that the stimuli for the increases in the enzyme activity cause additional alterations in the liver cell and these unknown changes may be essential for the subsequent formation of DNA.

Acknowledgement

This work was supported by the National Cancer Institute and the American Cancer Society.

References

[1] M. Fujioka, M. Koga and I. Lieberman, J. Biol. Chem. 238 (1963) 3401.
[2] I. Lieberman, In Vitro 6 (1970) 46.
[3] J. Short, R.F. Brown, A. Husakova, J.R. Gilbertson, R. Zemel and I. Lieberman, J. Biol. Chem. 247 (1972) 1757.
[4] J. Short, N.B. Armstrong, R. Zemel and I. Lieberman, Biochem. Biophys. Res. Commun. 50 (1973) 430.
[5] D. Russell and S.H. Snyder, Proc. Natl. Acad. Sci. U.S. 60 (1968) 1420.
[6] J. Jänne and A. Raina, Acta Chem. Scand. 22 (1968) 1349.
[7] T.R. Schrock, N.J. Oakman and N.L.R. Bucher, Biochim. Biophys. Acta 204 (1970) 564.
[8] E. Hölttä and J. Jänne, FEBS Letters 23 (1972) 117.
[9] J. Jänne and H.G. Williams-Ashman, J. Biol. Chem. 246 (1971) 1725.
[10] S.H. Snyder and D.H. Russell, Federation Proc. 29 (1970) 1575.
[11] J.M. Pekarthy, J. Short, A.I. Lansing and I. Lieberman, J. Biol. Chem. 247 (1972) 767.
[12] J.P. MacManus, D.J. Franks, T. Youdale and B.M. Braceland, Biochem. Biophys. Res. Commun. 49 (1972) 1201.