Vol. 93, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1980 Pages 661-667

BIPHASIC INCREASE IN ORNITHINE DECARBOXYLASE AND ITS RELATIONSHIP WITH THYMIDINE KINASE AND DNA SYNTHESIS IN LIVER: EFFECTS OF DIETARY PROTEIN AND AMINO ACID MIXTURE

Ishtiaq Mahmud, Mineko Asanagi and Yoshiaki Miura

Department of Biochemistry, Chiba University
School of Medicine, Chiba 280, Japan

Received December 1,1979

Summary: In rats, feeding protein free diet for 4 days followed by starvation and then high protein diet induced a biphasic ornithine decarboxylase (EC 4.1.1.17) activity, prolonged thymidine kinase (EC 2.7.1.21) activity and DNA synthesis. In contrast feeding a diet containing casein-equivalent amino acid mixture induced a monophasic ornithine decarboxylase activity, short-lived thymidine kinase activity and DNA synthesis. To maintain prolonged thymidine kinase activity and DNA synthesis high protein diet must be given in the early part of the prereplicative period.

INTRODUCTION: Multiplication of hepatocytes can be induced in rats by partial hepatectomy, infusion of hormones (1) or by suitable levels of dietary protein or amino acid (2). DNA synthesis begins in 8 to 12 hours (3), the exact time depending on the method employed for induction. It is now believed that synthesis of DNA in nucleus requires a series of biochemical changes that takes place during the prereplicative period, changes in RNA and protein metabolism are observed in the period from 8 to 12 hours after the stimulus (4).

A relationship between early induction of ornithine decarboxylase (ODC) activity and cell growth rate has been found by several workers in a wide variety of tissues under different conditions. ODC catalyzes ornithine decarboxylation to yield putrecine. It is the first step and key enzyme of the metabolic pathway leading to polyamines spermidine and spermine

Abbreviations: ODC, ornithine decarboxylase; t-RNA, transfer RNA; r-RNA, ribosomal RNA; Tdr kinase, thymidine kinase.

0006-291X/80/070661-07$01.00/0

synthesis (5). Although the exact function and mechanism of action of the polyamines are not clearly understood, some studies suggest that polyamines interact with nucleic acids inside the cell and can act at the transcriptional and translational levels (6,7). Involvement of polyamines in methylation of t-RNA and r-RNA was also demonstrated (8,9).

Initiation of DNA synthesis requires the presence of deoxyribonucleoside triphosphates. Studies on the levels of the enzymes involved in the biosynthesis of these precursors have revealed that some of them, particularly thymidine kinase, increase in activity at the same time, or possibily just before DNA replication (10).

Previously we reported that protein (casein) was specifically necessary for prolonged DNA synthesis in rat liver, when a high protein diet is given after a period of protein free diet followed by starvation (11). An equivalent amount of amino acid mixture could not replace casein in prolonging the DNA synthesis. The present paper deals with the changes in the enzymes of the prereplicative period after feeding a diet containing casein or amino acid mixture to the starved rats. We observed a biphasic increase in ODC activity when 36% casein diet was fed but a monophasic increase when amino acid diet was given. Of a 24 hr feeding period, when casein diet was fed at the begining followed by amino acid diet, prolonged thymidine kinase activity and DNA synthesis was observed, but, when amino acid diet was fed at the begining followed by casein diet, thymidine kinase activity and DNA synthesis were short lived.

MATERIALS AND METHODS: Animals and diets-Female Donryu strain rats weigning 140-150g were obtained from Nippon Rat Co. Urawa, Saitama. Rats were maintained on normal laboratory chow for a week and then given protein free diet for 4 days followed by starvation of 8 hr.

Food and water was always given *ad libitum*. The animals were kept at 23 ± 0.5°C and the room was lighted from 9 AM to 9 PM. The period in which high protein and amino acid diet was given, was mentioned in the legend of each experiment. The compositions of the protein free diet, high protein diet and amino acid diet were given in our previous paper (11). DL-(1-^{14}C) ornithine (58 mci/m mole), (2-^{14}C) deoxy thymidine (59 mci/m mole) and methyl-(^{3}H) thymidine 5 ci/m mole were purchased from the Radiochemical Center, Amersham, England. L-Amino acids were kindly donated by Tanabe Pharmaceutical Research Laboratory, Toda, Saitama, Japan.

ODC activity was mesaured in the supernatant fraction of a liver homogenate obtained by centrifugation at 105,000xg for 60 min. The assay method of Fukui et al.(12) was used with minor modification. One gram liver was homogenized in 9 vol of 50 mM Tris-HCl buffer, pH 7.5, containing 5 mM dithiothreitol. The radioassay mixture contained 1.3 ml of buffer mixture, 0.5 ml of enzyme and 0.5 µci of (1-^{14}C) ornithine in 0.2 ml of 0.5 mM DL-ornithine solution. The buffer mixture consisted of 80 mM Tris-HCl pH 2.5, 0.08 mM Pyridoxal phosphate, 1.6 mM EDTA and 4 mM dithiothreitol. The radioassay mixture was incubated at 37°C for 30 min. and the reaction was terminated by adding 1 ml of 2 M citric acid. Thymidine kinase was assayed in supernatant fraction of a liver homogenate obtained by centrifugation at 105,000xg for 60 min. The method used by Fukui (13) was used with slight modifications. Liver was homogenized in 0.2 M Tris-HCl buffer pH 8 assay mixture containing 0.1 mM deoxythymidine (2-^{14}C) (0.167µci), 10 mM ATP, 10 mM $MgCl_2$ $6H_2O$ and 100 mM Tris-HCl buffer was incubated for 30 min. at 37°C with 100 µl of enzyme solution in a total volume of 500 µl. DNA synthesis was estimated by the method of Verley's (14) as incorporation of (^{3}H)-thymidine into DNA of liver slices. Detail procedure of the estimation was given in the previous paper (11).

RESULTS: Induction of ODC-Changes in ODC activities in the liver of rats fed on 36% casein diet and 36% amino acid diet are shown in (Fig.1). With casein diet a biphasic increase in the activity was obtained with peaks at 3 and 11 hr, the first peak being higher than the second. On the contrary, with amino acid diet there was only a single peak at 3 hr, the extent of the induction at 3hr was the same as that of casein diet, but thereafter the activity gradually decreased.

Induction of Thymidine kinase—The time period for effectively inducing thymidine kinase in starved rats by alternately feeding casein and amino acid containing diet in a 24 hr feeding period - were shown in (Fig.2). The diet containing amino acids which are readily absorbed from the intestine was given at the begining

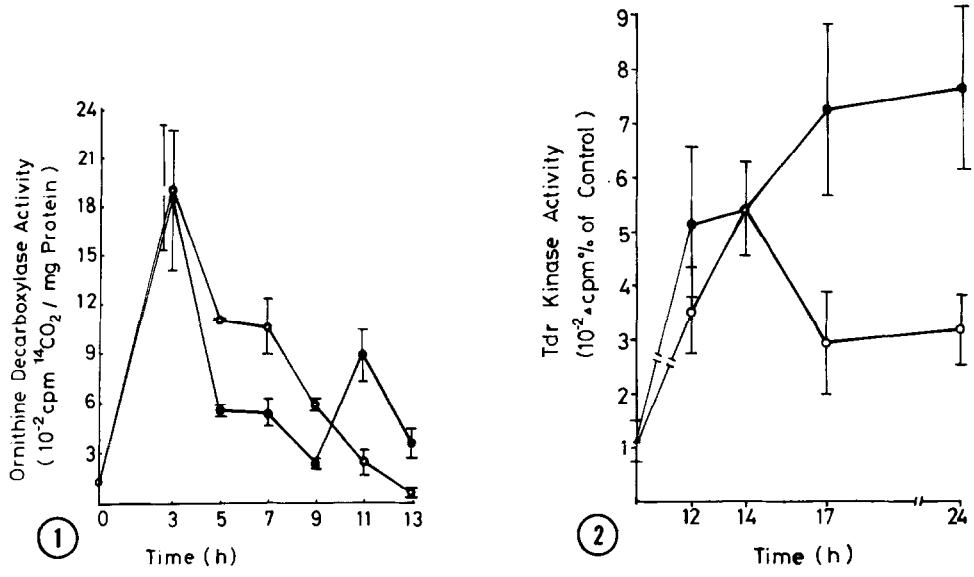

Fig.1. The effect of diet containing either casein or casein equivalent amino acid mixture on the induction of ornithine decarboxylase.

The rats after 4 days of protein free diet and 8 hr starvation were given either 36% casein diet (●) or 36% casein equivalent amino acid mixture diet (○) for the period indicated in the fig. Each point represents the average of results for 4 to 7 rats.

Fig.2. The effect of casein or amino acid containing diet on the induction of Thymidine kinase when fed alternately for a period of 24 hr.

Rats were fed protein free diet and starved as mentioned in the legend of Fig.1. Casein diet (●) was given for the indicated time followed by amino acid diet for the rest of the 24 hr period. Conversely amino acid diet (○) was given for the indicated time followed by casein diet for the rest of the period. Rats were killed between 9 to 11 AM. Each point represents the average of results for 4 to 7 rats.

followed by casein diet for the rest of the feeding period, the enzyme activity decreased after 14 hr. But, when the feeding started with casein diet followed by amino acid diet, there was a steady increase in the enzyme activity throughout the whole feeding period.

Induction of DNA synthesis—DNA synthesis was also induced by alternately feeding casein and amino acid diet and *vice versa* as in the case of thymidine kinase. Thus in a 24 hr feeding period, when feeding was started with amino acid diet followed by casein

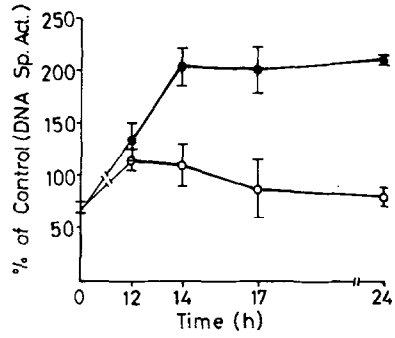

Fig.3. The effect of casein or amino acid containing diet on the induction of DNA synthesis when fed alternately for a period of 24 hr.

Rats were fed according to the method described in the legend for Fig.2. Casein diet is indicated by the closed circle (●) and the amino acid diet is indicated by the open circle (○). The rats were killed between 9 to 11 AM. Each point represents the average of results for 4 to 7 rats.

diet for the rest of the period, the induction continued only up to 12 hr and then decreased gradually to the basal level.(Fig.3) On the contrary when casein diet was fed at the begining DNA synthesis continued up to 14 hr and remained in the same high level for the remaining feeding period.

DISCUSSION: Although close relationship between the alterations of certain enzyme activities of the prereplicative period and DNA synthesis has been studied for a long time, the exact mechanism by which alterations in the enzyme activities causing the cell to proliferate is far from being clear. One of such enzymesis ODC, the relationship between its activity and macromolecular synthesis during cell cycle is well established in different tissues of different species. A monophasic increase in the activity of liver ODC in regenerating liver after partial hepatectomy was reported (15,16). In a careful time study Hölta and Jane (17) showed that increase in ODC activity after 70% hepatectomy is biphasic. Recently Lafarge *Frayssinet et al.(18) reported a triphasic increase in ODC activity in the cell cycle of *Euglena gracilis* Z synchronous culture. The prereplicative

elevation of ODC by different stimuli and subsequent DNA synthesis though seemed well corelated, but the exact mechanism for biphasic stimulation and the role played by the two peaks later in the DNA synthesis are not clearly understood. Gaza et al.(19) reported that probably two rises are induced by two different stimuli and perhaps the second rise is under translational control and does not require RNA synthesis and is dependent on the RNA that is formed during the period of initial elevation in activity.

Our data, of a biphasic increase in ODC by casein and a monophasic increase by amino acid diet, suggest that casein, a macromolecule, contains the stimuli for both peaks, and amino acid, a low molecular weight nutrient, is lacking the stimulus for the second peak. The necessity of protein in the early part of the feeding for biphasic induction of ODC observed by us, also implies that probably the second rise is under translational control and dependent on the RNA formed during the initial elevation.

Regarding the function played by the two peaks, there are reports of a possible relationship between the second peak and later formation of DNA (19). From our result it can be seen that the second peak of ODC has profound effect in prolonging the increased thymidine kinase activity and DNA synthesis in the later stage of the cell cycle.

However, at present we don't have any knowledge about the exact nature of the stimulus present in the casein diet for the second peak and how it is influencing the prolongation of increased thymidine kinase activity and DNA synthesis.

REFERENCES

1. Short,J., Brown,R.F., Husakova,A., Gilbertson,J.R., Zemel,R., and Lieberman,I. (1972) J.Biol. Chem. 247, 1757.

2. Short,J., Armstrong,N.B., Zemel,R., and Lieberman,I. (1973) Biochem. Biophys. Res. Commum. 50, 430-437.
3. Short,J., Armstrong,N.B., Gaza,D.J., and Lieberman,L. (1973) Liver Regeneration after Experimental Injury (Lesch,R. & Reutter,W., eds.) pp 296-309, Stratton Intercontinental Medical Book Corporation, N.Y.
4. Short,J., Armstrong,N.B., Kolitsky,M.A., Zemel,R., and Lieberman,L. (1974) Control of Proliferation in Animal Cells (Clarkson,B. & Baserga,R., eds.) pp 37-48, Cold Spring Harbor Laboratory, N.Y.
5. Williams-Ashman,H.G., Pegg,A.E., and Lockwood,D.H. (1969) Adv. Emzyme Regul. 7, 291-293.
6. Bachrach,U. (1973) Function of Naturally Occuring Polyamines Acadimic Press, N.Y.
7. Cohen,S.S. (1971) Introduction to the Polyamines Prentice Hall, N.Jersey
8. Pegg,A.E. (1971) Biochem. Biophys. Acta. 232, 630-642.
9. Sipe,J.E., Marshall Anderson,Jr.W., Remy,C.N., and Love,S.H. (1972) J.Bacteriol. 110, 81-89.
10. Bray,G., and Brent,T.P. (1972) Biochem. Biophys. Acta. 269, 184-189.
11. Mahmud,I., and Miura,Y. (1977) J.Biochem. 82, 911-913.
12. Fukui,N., Fujita,A., Ohtsuka,H., and Miura,Y. (1974) J. Biochem. 75, 867-873.
13. Fukui,N. (1971) J.Biochem. 69, 1076-1082.
14. Verley,W.G. (1973) Natl. Cancer Inst. Monograph. 38, 175-184.
15. Panko,W.B., and Kenney,F.T. (1971) Biochem. Biophys. Res. Commun. 43, 346-350.
16. Jänne,J., Raina,A., and Simes,M (1968) Biochem. Biophys. Acta. 166, 419-426.
17. Hölta,E., and Jänne,J. (1972) FEBS Letters 23, 117-120.
18. Lafarge- Frayssinet,C., Bertaux,O., Valencia,R., and Frayssinet,C. (1977) Biochem.Biophys. Acta. 539, 435-444.
19. Gaza,D.J., Short,J., and Lieberman,I. (1973) Biochem. Biophys. Res. Commun. 54, 1483-1487.