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INDUCTION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE BY HYDROCORTISONE IN RAT LIVER AND BRAIN AS A FUNCTION OF AGE

Ramesh Sharma# and S. K. Patnaik*

*Biochemistry Division, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221005, India

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Summary. The activity and induction pattern of phosphoenolpyruvate carboxykinase (PEPCK) in the liver and brain of young (6-), adult (30-) and old (90-weeks) male rats were studied. The activity of this enzyme increases in both tissues until adulthood and decreases gradually thereafter. Further, the activity of PEPCK is higher in the liver than the brain. Adrenalectomy decreases significantly the activity of this enzyme in the liver of rats of all ages. However, this treatment inhibits brain PEPCK in young and adult rats. Administration of hydrocortisone to adrenalectomized rats increases PEPCK in both tissues of young and adult rats. However, the magnitude of induction is higher in the young, as compared to the adult, rats. This hormone-mediated induction of the enzyme is actinomycin D-sensitive.

Introduction

Differentiation and development are programmed processes which occur due to sequential activation and repression of genes causing alterations in the levels of enzymes [1,2]. Phosphoenolpyruvate carboxykinase [PEPCK: GTP: oxaloacetate carboxylase (transphosphorylating) EC 4.1.1.32] is a key enzyme of gluconeogenesis [3], catalyzing the reversible decarboxylation of oxaloacetate to phosphoenolpyruvate. The enzyme has been found to be present in high concentrations in the liver and kidney cortex of all species [4] and in lung and brain tissues in some species [3]. The relative amounts of this enzyme in the mitochondrial and cytosol fractions of animal tissues vary greatly from one species to another. In rat liver, it is predominantly cytosolic [5]. It is the cytosolic PEPCK that responds to hormonal and dietary influences [6]. There are several reports on the induction of hepatic PEPCK by hydrocortisone [7,8]. However, information concerning age-related differential regulation of this enzyme by hydrocortisone in the liver and brain of rats is not available. The present paper describes the activity and induction pattern of liver and brain PEPCK by hydrocortisone in male rats of different ages.

Present address: Department of Life Sciences, Manipur University, Canchipur, Imphal-795003, India.

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Materials and Methods

Male albino rats of the Wistar strain (Rattus norvegicus albinus) of three different ages (6-, 30-, and 90-weeks) were used. They were maintained at 24 ± 2°C with a 12-h light period followed by a 12-h dark period. The rats were fed with a freshly prepared diet containing wheat flour and vitaminized milk-powder in the ratio of 4 : 1 in water with added table salt. The diet was supplemented with gram (Cicer arietinum) on alternate days. Tap water was supplied ad libitum. All the chemicals used were of analytical grade, and the biochemicals were purchased from Sigma Chemical Co., U.S.A.

Effects of adrenalectomy and hydrocortisone on PEPCK. Pilot experiments were undertaken to find out the time and dose dependence of the enzyme towards hydrocortisone in rats of various ages. Maximum response of the enzyme was obtained 3 days after hormone administration at a dose of 5.0 mg/100 g body wt. The rats of each age group were divided into four sets, each containing 4-5 rats. Set I rats served as the control. Rats of sets II, III, and IV were bilaterally adrenalectomized and were given 0.9% NaCl ad libitum instead of water for 10 days following adrenalectomy. On the 11th day, set II rats received 1.0 ml of 0.9% NaCl intraperitoneally (i.p.) instead of the hormone and these rats served as the control for the induction studies. Sets III and IV rats were given an i.p. dose of hydrocortisone (5.0 mg/100 g body wt., suspended in 1.0 ml of 0.9% NaCl) at a fixed time of the day (i.e. 5:00 pm) for 3 days. Set IV rats were also given actinomycin D (10.0 µg/100 g body wt., suspended in 1.0 ml of 0.9% NaCl), one h prior to each hydrocortisone injection. All the rats were killed 3 h after the final hormone injection and their liver and brain tissues were taken out, washed in normal saline, and blotted dry on filter paper.

Assay of PEPCK. A 20% (w/v) homogenate of the liver and brain was prepared in 10.0 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose. The homogenate was centrifuged for 30 min at 14,000 ×g at 0°C. PEPCK activity was measured according to the method of Ballard and Hanson [9] using a Beckman model DBGT spectrophotometer. The reaction mixture (final volume of 1.0 ml) contained the following components added in the order given: 100 µmol Imidazole-HCl buffer, pH 6.7; 50 µmol $NaHCO_3$; 1.25 µmol inosine 5'-diphosphate; 2.0 µmol $MnCl_2$; 2.0 µmol GSH; 20 units of malate dehydrogenase; 0.05 ml of a suitably diluted supernatant; 0.15 µmol NADH; and 1.5 µmol phosphoenolpyruvate. The protein content of the supernatant was determined [10] and the enzyme activity was expressed as units/mg protein. One unit of the enzyme is the amount that catalyzes 1.0 µmol of substrate/min at 25°C. The data were statistically analysed [11].

Results and Discussion

Our data show (Table I) that the activity of PEPCK of the liver and brain is significantly higher in adult rat as compared to those of the young and old rats. It is of interest to note that the observed changes in the level of PEPCK show similar trends with those of the isoenzymes of rat liver alanine aminotransferase (AAT) as a function of age. Although cytoplasmic-AAT participates in active gluconeogenesis, its activity decreases in old age [12]. A decrease in the activities of two other gluconeogenic enzymes (i.e., glucose-6-phosphatase and fructose-1,6-diphosphatase) in the liver of aging rats has been reported earlier [13]. The higher activity of PEPCK in the adult rat may be due to an increased metabolic activity of the animal during this phase of

TABLE I. PHOSPHOENOLPYRUVATE CARBOXYKINASE ACTIVITY IN THE LIVER AND BRAIN OF NORMAL MALE RATS OF DIFFERENT AGES

Age (weeks)	Enzyme activity*					
	Liver			Brain		
	Mean	S.D.	p	Mean	S.D.	p
6	30.00	± 2.40	<0.001	18.50	± 0.75	<0.01
30	47.60 (+59%)	± 1.20	<0.001	23.20 (+25%)	± 1.20	<0.05
90	28.20 (-41%)	± 1.30		19.80 (-15%)	± 0.80	

The data were collected from 4-5 animals of each age group. Standard deviation (S.D.) and the levels of significance (p) are given. Figures in parentheses are the difference of enzyme activity from that of previous phase in terms of per cent.
* units/mg protein.

its life-span [14]. The decrease in enzyme activity in old age may be correlated with a decrease in the rate of synthesis of corticoids as a function of increasing age of the rat [15] since this enzyme is regulated by corticosteroids [7]. It has also been suggested by Bentle and Lardy [16] that the rate of phosphoenolpyruvate synthesis in gluconeogenic tissues may be regulated by the availability of intracellular Fe^{2+} to the ferroactivator and PEPCK. The higher level of this enzyme in the liver as compared to the brain is correlated with the more pronounced gluconeogenic nature and higher level of ferroactivator protein [17] in the former.

Adrenalectomy decreases significantly the activity of liver PEPCK in rats of all ages (Fig. 1). However, this treatment also decreases the activity of the brain enzyme in young and adult rats. The decrease in the activity of PEPCK following adrenalectomy is significantly higher in the liver as compared to that of the brain. The per cent decrease in the activity of this enzyme following adrenalectomy is highest in the adult rat which is correlated with a higher endogenous level of this enzyme during this phase of its life-span. Administration of hydrocortisone to adrenalectomized rats increases significantly the activity of both the liver and brain PEPCK in young and adult rats, but not in the old rat (Fig. 1). The magnitude of induction in higher in young (2.5-fold) as compared to adult (1.8-fold) rats. The impairment of

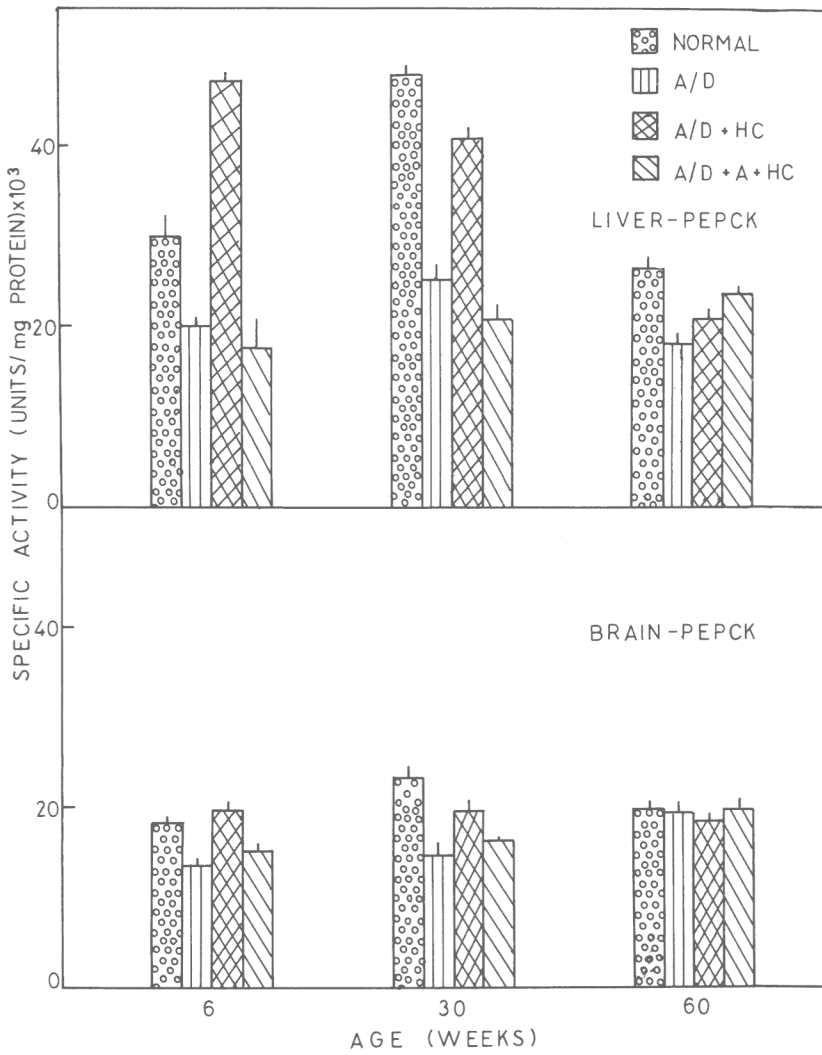

Fig. 1. Effects of adrenalectomy (A/D) and of treatment with hydrocortisone (HC) and actinomycin D (A) on the specific activity of phosphoenolpyruvate carboxykinase (PEPCK) in the liver and brain of male rats of various ages.

induction of PEPCK in old age may be due to a gradual loss in the level of hydrocortisone-receptors [18-20]. Another possibility for such a gradual decrease in the inducibility of PEPCK by hydrocortisone may be due to a gradual decrease in the responsiveness or derepression of the gene(s) responsible for the synthesis of this enzyme with increasing age of the rat. It has also been reported earlier that some of the non-histone chromosomal proteins (NHCP) undergo changes in old age [21], and it is well known that the hormone-receptor complex binds to the NHCP fraction of the chromatin. Hence, the reduction in NHCP fraction may also be responsible for the impaired induction of PEPCK in old age. A decrease in the degree of induction of cytoplasmic aspartate aminotransferase and two other gluconeogenic enzymes (i.e. glucose-6-phosphatase and fructose-1,6-diphosphatase) with advancing age of the rat has been reported earlier [13,22]. The induction of PEPCK by hydrocortisone in both tissues is actinomycin D-sensitive. Thus, it appears that the induction of this enzyme by the hormone occurs at the transcriptional level and is due to an increase in the synthesis of mRNA(s) for this enzyme. Iynedjian and Hanson [23,24] have also reported that glucocorticoid treatment causes a simultaneous stimulation of PEPCK-mRNA, its rate of synthesis, and also the total enzyme activity.

From the above mentioned findings, it may be concluded that the level and inducibility of PEPCK by hydrocortisone undergo specific changes during different phases of the life-span of the rat. Such alterations in the level of this enzyme may be due to regulatory changes in the corresponding gene(s) which are brough about by factors such as hormones according to a specific program [25].

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