SEX AND AGE DEPENDENT RESPONSE OF PYRUVATE KINASE OF THE BRAIN OF THE RAT TO ESTRADIOL AND TESTOSTERONE

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Received July 9, 1976

SUMMARY - Effects of estradiol and testosterone on pyruvate kinase of the cerebral hemisphere of 7-, 38- and 78-week old male and female rats were studied. The activity of the enzyme is highest in immature rats of both the sexes and decreases thereafter. Castration decreases its activity in all the ages of both the sexes except in adult males. Estradiol induces the enzyme in castrated rats of both the sexes of the three ages. However, the pattern of induction differs with dose and sex of the animal. Testosterone has no striking effect on the enzyme level of both sexes. These findings are discussed in relation to the differential responsiveness of genes at various phases of the life span of the rat.

INTRODUCTION - Pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40; PK) catalyses the conversion of phosphoenol pyruvate, and is a rate limiting enzyme of the glycolytic path. Its role as a pacemaker in the central nervous system has been reported (1). An alteration in the hormonal balance causes a short or long term change in the catalytic property of PK of the liver (2, 3). Age-related changes in the induction pattern of many enzymes of the rat by various hormones have been reported from this laboratory (4, 5) and elsewhere (6). We report here both sex and age dependent response of PK of the brain of rats to estradiol and testosterone.

MATERIALS AND METHODS - Immature (7-), adult (38-) and old (78-week) male and female rats maintained in standard laboratory conditions

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Abbreviations: PK, pyruvate kinase; LDH, lactate dehydrogenase.

were used. The rats of the three ages of each sex were divided into six sets. Set I served as normal control. Set II-IV were bilaterally castrated and kept for 21 days before experimentation. On 22nd day, the rats of sets III and IV were given 50 and 100 μg estradiol/100 gm. body wt., respectively, and the rats of sets V and VI were given 50 and 100 μg testosterone/100 gm. body wt. The hormones were dissolved in 10% normal saline containing 10% ethanol and administered intraperitoneally. The castrated rats of set II served as control for the hormone treated animals and were given normal saline instead of hormone. All the injections were given at 4 P.M., and the animals were sacrificed at 8 P.M. by cervical dislocation. The brain (cerebral hemispheres) was dissected out, washed in cold normal saline and minced. A 5% homogenate was prepared in a medium containing 0.15 M KCl, 0.05 M $MgSO_4$ and 0.001 M EDTA using a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 14,000 x g for 20 min. The supernatant was used for the assay of the enzyme (7). The protein content of the supernatant was determined (8) and the enzyme activity was expressed as units/mg protein. Each set of data was collected from 4-6 rats, and statistically analyzed.

RESULTS AND DISCUSSION - Our data show that the PK level is highest in the immature (7-week) rat and decreases thereafter. The level in the adult female is lower than in adult males. However, the level in old males is lower than in old females. The decrease in PK level with increasing age may be correlated with a gradual decrease in the respiratory rate of the brain (9). The brain is highly aerobic in nature. The decrease in PK level in old age may decrease the capacity of the organ to withstand anaerobic condition which is of definite disadvantage to the organ. This is supported by the earlier finding that the activity of lactate dehydrogenase (EC L-

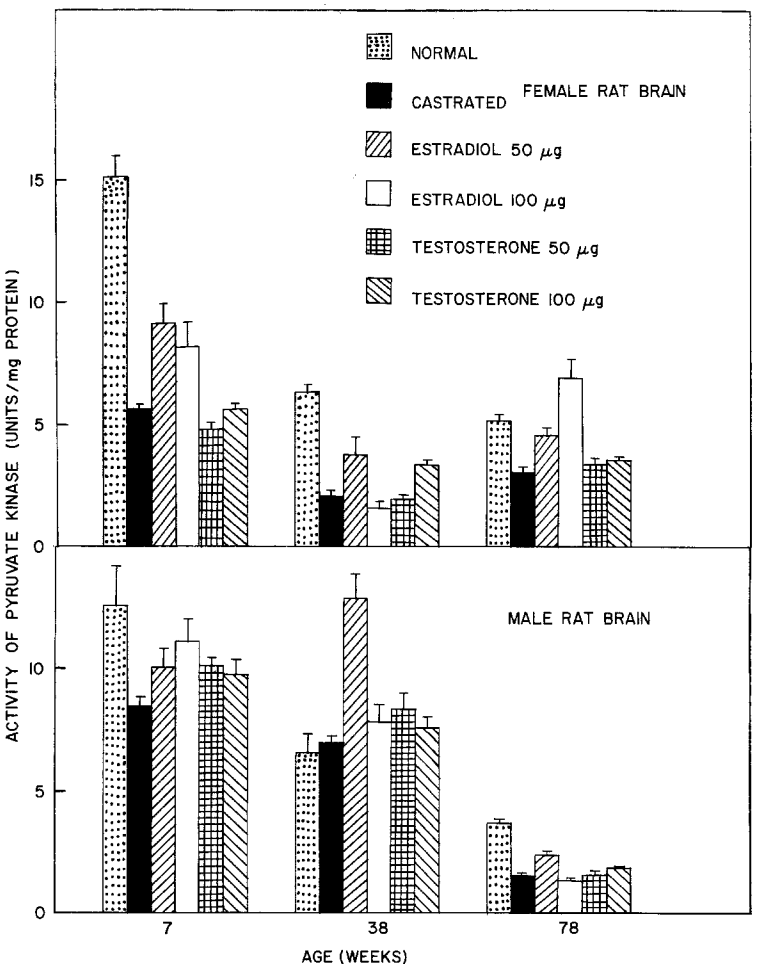

Fig. 1. Effect of estradiol and testosterone on the specific activity of pyruvate kinase of cerebral hemisphere of male and female rats.

lactate:NAD oxidoreductase, EC 1.1.1.27) and oxygen consumption of the brain of rats is lower in old rats (9, 10). The decrease in the enzyme level and the differences in the two sexes in old age may also be due to the loss of neurons or a decrease in the rate of protein synthesis in old age. This is supported by the finding that the polyribosome content of the brain is lower in old age (11).

Castration caused a decrease in the enzyme level of all the

age groups of both the sexes except in the adult male (Fig. 1). It is difficult to explain why castration has no effect on the enzyme in adult male rats. This may be due to hypertrophy of the adrenal in the adult to compensate for the hormone. The data show that administration of 50 μg estradiol induces the enzyme in all the age groups of spayed rats. The induction is maximum in the adult, and decreases in the old. The retention of estradiol in the brain and a decrease in the level of estradiol-binding protein in old female rats have been reported (12, 13). Estradiol 100 μg induces the enzyme in young and old, whereas it decreases its level in the adult. The induction is higher in the old than in the young. There does not appear to be a corresponding increase in induction when the dose is doubled.

In orchidectomized male rats, administration of 50 μg estradiol shows the same induction pattern as in ovariectomized female rats. A decrease in induction in old age may be due to a decrease in the estradiol-binding protein with increasing age. Such specific binding of estradiol in cytosol of the brain of castrated male rats has been reported (14). 100 μg estradiol induces the enzyme in the young and has no effect in adult and old male rats. A similar decrease in the induction of tyrosine aminotransferase by higher dose of hydrocortisone in old age has been reported (15).

Even though castration causes a decrease in the level of PK in both male and female rats, administration of either 50 or 100 μg of testosterone does not induce PK significantly (Fig. 1).

It is of much significance that estradiol induces PK of the brain not only in the female, but also in the male. This induction varies with age of the rat. The slight induction in the adult male by testosterone may be due to the transformations of testosterone to

estradiol (16). It has been reported that sex steroids induce PK in accessory sex organs by stimulating gene expression (17). Thus, our finding of sex- and age-dependent induction of PK in the brain of rats by estradiol is consistent with the model proposed for aging (18) according to which regulatory changes in the expression of genes brought about by various factors like hormones may account for the changes in the levels of enzymes seen at various ages of the life span.

ACKNOWLEDGEMENTS - This research was supported by research grants from Nuffield Foundation, England, and PL-480 (FG-In-540) to M.S.K. G.B.N.C. thanks the U.G.C. for a research fellowship.

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