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Rat brain glycolysis regulation by estradiol-17β

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The effect of estradiol-17β on the activities of glycolytic enzymes from female rat brain was studied. The following enzymes were examined: hexokinase (HK, EC 2.7.1.1), phosphofructokinase (PFK, EC 2.7.1.11), aldolase (EC 4.1.2.13), glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), phosphoglycerate kinase (EC 2.7.2.3), phosphoglycerate mutase (EC 2.7.5.3), enolase (EC 4.2.1.11) and pyruvate kinase (PK, EC 2.7.1.40). The activities of HK (soluble and membrane-bound), PFK and PK were increased after 4 h of hormone treatment, while the others remained constant. The changes in activity were not seen in the presence of actinomycin D. The significant rise of the activities of the key glycolytic enzymes was also observed in the cell culture of mouse neuroblastoma C1300 treated with hormone. Only three of the studied isozymes, namely, HKII, B_4 and K_4 were found to be estradiol-sensitive for HK, PFK and PK, respectively. The results obtained suggest that rat brain glycolysis regulation by estradiol is carried out in neurons due to definite isozymes induction.

Introduction

Female sex steroid hormones appear to exert a variety of effects on the nervous system. These effects range from neuroendocrine regulation to alterations in the electrical activity of neural cells and finally to an influence on mood, motivation and behavior pattern. The responses of nervous tissue to the action of steroid hormones are mediated by intercellular receptors localized in different regions of the brain. Specific steroid receptors bind the hormone molecules and carry them to the cell nuclear compartment, where genomic interactions result in initiating the synthesis of structural and functional proteins. The steroid hormones, thus affecting the metabolism of neurons, lead to the alterations in the processes of neurotransmission. In view of a possible modulation of sexual behavior two subjects have long been a matter of interest of researchers in this field. These are the steroid administration of the level of receptors of neurotransmitters and the hormone influence on the activities of enzymes participating in the synthesis and degradation of neurotransmitters. So, the activities of cholinacetyltransferase, monoamine oxydase and tyrosine hydroxylase are known to be modified by estradiol in rat brain [1–3]. At the same time, the hormonal effect on the enzymes of the other metabolic pathways is less known. Only a few attempts have been made to find estrogen regulation of enzymes taking part in energy metabolism and requiring energy supply. It has been reported that the activities of brain lactate dehydrogenase, isocytrate dehydrogenase, malate dehydrogenase and creatine kinase are increased after estradiol treatment [4,5]. Therefore, it seems interesting to study the influence of estradiol on glycolysis, which is the first step of energy metabolism. It plays an important role in nervous tissue since more than 90% of glucose, being the main energy source, is utilized via this metabolic pathway.

The aim of the present study was to test the effect of estradiol-17β on the activities of glycolytic enzymes in both rat brain and cell culture of mouse neuroblastoma C1300. In order to confirm hormonal induction of the key glycolytic enzymes activated after estradiol injection we have used actinomycin D as an inhibitor of protein synthesis. We were also interested in finding out which isozyme of each inducable enzyme is more sensitive to hormonal action.

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Materials and Methods

Materials. Common chemicals, estradiol-17β, actinomycin D and Eagle's medium were obtained from Serva, special chemicals and enzymes from Boehringer, and ampholytes from LKB. Mouse neuroblastoma C1300 (clone 2Na) was taken from Institute of Cytology, Academy of Sciences of U.S.S.R., Leningrad.

Hormonal administration. Estradiol-17β (30 μg/100 g body wt.) in propylene glycol was administrated to female white rats (130–150 g body wt.) as intraperitoneal injection. Controls received propylene glycol only. The animals were killed 4 h after hormone treatment. Actinomycin D was injected an hour before estradiol administration.

Tissue preparation. Brain was removed and homogenized in 10 volumes of 50 mM Tris-HCl (pH 7.5) at 1500 rev/min for 1 min; cytosols were obtained by centrifugation at 17000 × *g* for 30 min at 4°C. Soluble and membrane-bound fractions of HK were prepared according to the method of Kellog et al. [6] and cytosol for the PFK assay was prepared in 50 mM sodium phosphate (pH 8.0). Protein was estimated by the method of Bradford [7] using bovine serum albumin as a standard.

Cultured cell line. Cells of mouse neuroblastoma C1300 were grown in modified Eagle's medium with 10% bovine serum in Falcon flasks or Petri dishes at 37°C for 40 h. 3.5 h, which corresponds to the maximal activity of key glycolytic enzymes [8], before the above-mentioned procedure estradiol-17β (25, 50 and 100 μg/ml) dissolved in 95% ethanol was added ex tempore to the cultured medium. Cultured cells were suspended in 0.25% trypsin solution, collected and removed twice in an EDTA ice solution. Cell suspension was treated with ultrasonic disintegrator in 50 mM Tris-HCl (pH 7.5) at 44 kHz and 4°C for 30 s. Control culture was kept in normal growth medium without hormone.

Enzymatic assays. Enzymatic activities were measured spectrophotometrically in 0.6 ml of assay medium at 240 or 340 nm for 1–3 min at 25°C in a spectrophotometer Specord M 40 (Carl Zeiss, Jena). HK, PFK, phosphoglycerate mutase and PK assays were carried out according to the methods described by Kostanyan et al. [8] and Nazaryan et al. [9]. Enolase activity was determined following Baranowski and Wolna [10]. As to aldolase, glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase we have used the methods of Bergmeyer [11].

Enzymatic activity was expressed as μmol of product per 1 mg of protein for 1 min.

Electrofocusing. Electrofocusing was performed in 5% polyacrylamide gel (PAAG) using carrier ampholytes in the pH range 3.5–10 according to the procedure of Winter et al. [12] with some modifications. Separation was made at 3.5 W of constant power, 0.5 mA of initial current and 10°C for 6 h. 5-mm wide gel strips were cut and incubated in 1 ml of enzyme assay medium at 37°C for 1 h and enzymatic activity was estimated in the medium obtained. pH gradient determination was carried out by the same procedure in the glass distilled water.

The statistical significance of differences between mean values was determined by the Student's unpaired *t*-test.

Results and Discussion

Effect of estradiol-17β on the activities of glycolytic enzymes from rat brain

The values of HK (soluble and membrane-bound), PFK and PK activities in brain from treated rats and controls are represented in Table I. The activities of all enzymes are increased after 4 h of estradiol-17β administration. The lowest change in activity is observed in the membrane-bound fraction of HK (19%) and the highest is found for PFK (84%). It is known that brain HK is inhibited by a physiological concentration of glucose 6-phosphate [13] and, therefore, under normal conditions the activity of this enzyme is usually depressed. The K_m value for ATP of membrane-bound HK is 3-fold lower and K_i for glucose 6-phosphate is 5-fold higher than that of the soluble ones. So, even a

TABLE I

Activities of key glycolytic enzymes in brain from rats treated with estradiol-17β and actinomycin D + estradiol-17β and controls

Enzymatic activity is given in μmol/mg per min and expressed as the mean ± S.D. * $P < 0.05$ ($n = 10$).

	Soluble HK	Membrane-bound HK	PFK	PK
Control	0.054 ± 0.009	0.174 ± 0.002	1.32 ± 0.02	1.88 ± 0.07
Estradiol-17β	0.075 ± 0.005 *	0.208 ± 0.006 *	2.45 ± 0.09 *	3.10 ± 0.06 *
Actinomycin D + estradiol-17β	0.053 ± 0.007	0.174 ± 0.001	1.31 ± 0.05	1.96 ± 0.04

small rise in the activity of the bound form could be enough for a significant increase in the rate of glucose phosphorylation [14].

It should be noted that the increase in PFK activity can not be related to allosteric activation since the assay was carried out at pH 8.0, when this enzyme is submitted to Michaelis' kinetics. Regarding the alterations in the activities of soluble HK and PK it was shown that their values were 38% and 60.6%, respectively.

At the same time we have found that the activities of aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase and enolase, the reactions of which are close to equilibrium, were not changed by estradiol (Table II).

Taking into account that the maximal rise in the rate of total protein synthesis is detected by 4 h of estradiol administration [15], we have supposed that the activation of the key glycolytic enzymes could be the result of hormonal induction. This was supported by the fact that some rat brain enzymes, for example, lactate dehydrogenase, acetylcholinesterase and creatine kinase are induced by estradiol [1,5,16]. In order to test our assumption, we have used actinomycin D to inactivate the DNA matrix and to block the process of transcription. There was no enzymatic activity change in the case of the animals pretreated with actinomycin D before they were treated with hormone (Table I). The results obtained confirm the activation of the studied enzymes using their synthesis de novo. Moreover, our findings are consistent with the observations of Baquer and McLean, concerning estradiol induction of HK, PFK and PK in rat uterus [17]. This can indicate a common mechanism of glycolysis regulation taking place in target tissues. The fact of estradiol induction of the key glycolytic enzymes only is interpreted by us in the following way. As glycolysis is mainly controlled by three kinases stages the rise of the activities of HK, PFK and PK could be enough for activation of the whole glycolytic pathway.

In this aspect it also of interest that the similar triple control of glycolysis by ATP exists in CNS. Since the ATP/ADP ratio shows the intensity of energetic metabolism in brain cells the glycolysis rate is controlled depending on the concentrations of these metabolites. Perhaps, the variations of the physiological concentration of estradiol can also regulate the glycolytic pathway requiring additional energy and/or metabolites for neurotransmission and thus facilitating the development of sexual behavior. It should be also noted that our observations are consistent with those of Weber et al. [18,19], concerning insulin induction of liver HK, PFK and PK. The authors have suggested that in this case glycolysis is controlled by the use of a 'functional genomic unit' like operon in procaryotes. However, later some investigations by Sillero et al. [20] and Takeda et al. [21] have refuted the hypothesis of Weber.

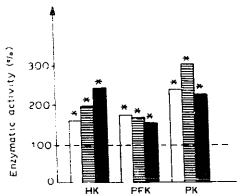

Fig. 1. Activities of key glycolytic enzymes in cell culture of mouse neuroblastoma C1300 after estradiol-17β treatment. 100% is given as a control. Hormone concentrations are: open bars, 25 μg/ml; hatched bars, 50 μg/ml and closed bars, 100 μg/ml. * $P < 0.05$ ($n = 6$).

Effect of estradiol-17β on the activities of the key glycolytic enzymes in the cell culture

As long as estradiol receptors were discovered in the neurons but not in the glia [22] we have assumed that glycolytic pathway regulation by estradiol detected in rat brain occurs only in neurons. The functional advantages of such a differentiated regulation are indisputable since it may effect immediately the energy requirement of neurotransmission. In order to confirm our conjecture we have tested the influence of estradiol-17β on the activities of the key glycolytic enzymes in the cell culture of mouse neuroblastoma C1300. This culture has many principal characteristics of differenti-

TABLE II

Activities of glycolytic enzymes in brain from rats treated with Estradiol-17β and controls

Enzymatic activity is given as described in Table I.

	Aldolase	Glyceraldehyde-3-phosphate dehydrogenase	Phosphoglycerate kinase	Phosphoglycerate mutase	Enolase
Control	0.73 ± 0.02	0.139 ± 0.04	0.72 ± 0.08	1.86 ± 0.03	1.01 ± 0.03
Estradiol-17β	0.75 ± 0.02	0.146 ± 0.08	0.78 ± 0.09	1.88 ± 0.07	1.03 ± 0.01

ated neurons, such as typical cell morphology, the ability for action potential generation, the possibility of the synthesis of neurotransmitters etc. As shown in Fig. 1, the significant rise of HK, PFK and PK activities was found in the cell culture after hormone administration. When we used a hormone concentration of 25 μg/ml, HK, PFK and PK activities were increased correspondingly by 60.5; 84.4 and 140.8%, while in the case of 50 μg/ml they were increased by 97.3; 72.2 and 211%. Finally, 100 μg/ml of estradiol caused the changes of enzymatic activity, which were 147.5; 60.3 and 134%, respectively. It is obvious that the changes obtained in activity are more expressed than the similar alterations observed in the whole rat brain. The differences in results of in vivo and in vitro experiments could be explained by the absence in the cell culture of many factors securing the homeostasis of brain cells.

Thus, glycolysis activation by estradiol in the cell culture of mouse neuroblastoma C1300 used as a neuron model lead us to the following conclusion. Estradiol regulation of the brain glycolytic pathway, realized by hormonal induction of regulatory enzymes, takes place in neuronal cells.

Effect of estradiol-17β on the isozymes' pattern of the key glycolytic enzymes from rat brain

In order to study the hormone-sensitivity of brain HK, PFK and PK isozymes inducable enzymes from treated rats and controls were separated by analytical electrofocusing in PAAG using carrier ampholytes in the pH range 3.5–10. Fig. 2a and Fig. 3a demonstrate two peaks obtained of HK activity in both soluble and membrane-bound fractions with pH values of 4.9 and 6.1. It has been reported that in mammalian brain only two isozymes of HK, termed HKI and HKII, were found and the pI value of the first predominant one was determinated as 6.2 [23]. By this reason we have identified peaks detected as HKII and HKI, respectively. As seen in Fig. 2b and Fig. 3b estradiol induced

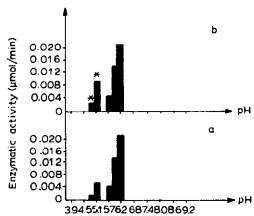

Fig. 3. Activity of membrane-bound HK in brain from controls (a) and rats treated with estradiol-17β (b) after electrofocusing in PAAG using carrier ampholytes in the range 3.5–10. * $P < 0.05$ ($n = 6$).

a rise of the minor component, whereas the enzymatic activity of the major component remained unchanged. These results agree with data of Katzen [24] and Kaur et al. [25], who have described the same HK isozyme response to insulin action. It has been supposed that HKII activity was increased by specific insulin activation in contrast with our conclusion of estradiol induction as a possible mechanism of enzyme activity regulation. Furthermore, the presence of HKII in different tissues correlates with their insulin-sensitivity [24]. Probably, such a correlation between this isozyme and estradiol-sensitivity of target tissues also exists. Simultaneously, at present we have no other experimental evidence, except the observation of brain HK.

It is not surprising that there are no differences in the soluble and membrane-bound HK isozyme patterns, excluding enzymatic activity values, as many observations indicate the identity of brain HK isozymes [6,14]. The fact that the total activity of membrane-bound HK is higher than that of soluble HK one is accounted for by the predominant localization of brain HK on mitochondrial membranes [26].

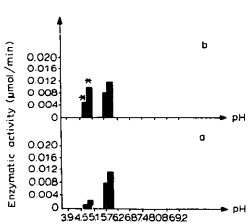

Fig. 2. Activity of soluble HK in brain from controls (a) and rats treated with estradiol-17β (b) after electrofocusing in PAAG using carrier ampholytes in the range 3.5–10. * $P < 0.05$ ($n = 6$).

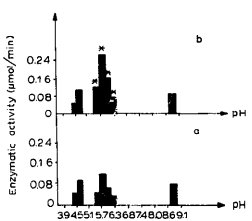

Fig. 4. Activity of PFK in brain from controls (a) and rats treated with estradiol-17β (b) after electrofocusing in PAAG using carrier ampholytes in the range 3.5–10. * $P < 0.05$ ($n = 6$).

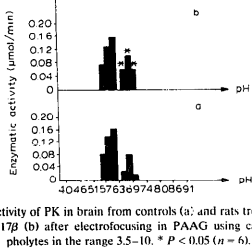

Fig. 5. Activity of PK in brain from controls (a) and rats treated with estradiol-17β (b) after electrofocusing in PAAG using carrier ampholytes in the range 3.5–10. * $P < 0.05$ ($n = 6$).

Fig. 4a shows three pools of PFK activity with pH values of 4.5, 5.8 and 8.5. The majority of the data, concerning the brain PFK isozymes pattern, indicate the presence of M, B and L subunits [27–30]. According to results represented by Foe et al. [27] and Vora et al. [28], the B form of the enzyme has an intermediate position by negative charge between the L and M forms. So, the pools obtained were designated as L_4, B_2 and M_4 isozymes, respectively. As to hybrid forms, theoretically, one can observe them as separate peaks. On the other hand, PFK subunits are close by charge value and, probably, the pools of activities of homo- and heterotetramers overlap each other. And, therefore, we have discovered three peaks only. It is especially evident in the range of the second pool as long as in mammalian brain only B subunits predominate over the other subunits [27,28]. It is interesting to note that only the B form of PFK was found to be estradiol-responsive, as demonstrated in Fig. 4b. At the same time, being an allosteric enzyme like M_4 and L_4, B_4 is the most sensitive to the action of allosteric effectors [28]. Perhaps, this isozyme plays an important role in PFK activation by allosteric regulation as well as by estradiol induction.

Finally, we have detected two peaks of PK activity with pH values of 5.7 and 6.5 (Fig. 5a). According to the data of Tolle et al. [31] only M_4 and K_4 isozymes occur in the rat brain and the former is known to be the prevalent component in the PK isozymes pattern. It has been reported that the pI value of mammalian brain M and K form of the enzyme vary in the range of 6.1–8.9 and 6.7–8.3, correspondingly [32]. To obtain additional information we have used purified PK from brain and muscle as controls (data not shown), due to which peaks of PK activity were identified as M_4 and K_4, respectively. Fig. 5b demonstrates the significant increase of K_4 isozyme activity following hormone treatment, whereas M_4 is insensitive to estradiol action. Our results agree with observations by Strandholm et al. [33], who demonstrated estradiol induction of K subunits in the rat uterus in contrast with other data, confirming the hormone-sensitivity of L subunits only [20,21,34].

Thus, we can conclude that estradiol regulation of brain glycolysis is carried out in neurons by hormonal induction of definite HK, PFK and PK isozymes, namely, HKII, B_4 and K_4. In this aspect it should be noted that Kaye [5] and Nagy et al. [16] have found estradiol induction of only B and M subunits of brain creatine kinase and lactate dehydrogenase, respectively. It seems probable that only definite isozymes can response to the action of the different factors like hormones and allosteric activators.

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