BIOCHEMICAL ASPECTS IN THE EXPERIMENTAL BARBITAL DEPENDENCE IV
—ITS EFFECT ON THE HEXOKINASE AND PHOSPHOFRUCTOKINASE ACTIVITIES IN THE RAT BRAIN—

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Abstract—Effects of barbital dosing on the neural hexokinase (EC 2.7.1.1) and phosphofructokinase (EC 2.7.2.11) activities in the rat, were examined. The barbital dependence in the rat was acquired by giving the barbital-admixed food. These two enzyme activities were investigated in the following three dissected portions of the brain: cerebral cortex, brain stem and cerebellum. The both enzyme activities in these three portions were depressed by the barbital dosing, while them being increased at the early stage of its withdrawal. From these results, it is considered that the measurements of hexokinase and phosphofructokinase activities probably give a possibility to estimate the degree of barbital dependence and its withdrawal, and that these two enzyme activities can be good incees in these conditions.
Key words: barbital dependence, withdrawal, hexokinase, phosphofructokinase, rat.

INTRODUCTION

It was reported that the carbohydrate metabolism could be one of the good incees in the neural activity, and that some drugs producing their dependences affected the cerebral carbohydrate utilization (Mukherje, 1980). Barbital intoxication was also reported to affect the cerebral carbohydrate metabolism (Yanaura et al. 1983). According to Yanaura et al. (1982), barbital depresses the central energy metabolism and carbohydrate metabolism, and this change causes a decrease of the rectal temperature in the rat. Its withdrawal from the long term dosing of barbital causes an increase of rectal temperature, with this increase being considered to be due to the changes of basic metabolisms including glycometabolism, energy metabolism and so on.

As to the rate-limiting enzymes in glycolysis, it has been known that hexokinase
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(EC 2.7.1.1) plays a role of rough adjustment, while phosphofructokinase (EC 2.7.2.11) plays another role of minute adjustment in glycolysis (Uj, 1977). In the central nervous system, hexokinase, phosphofructokinase and other kinases possess the following characteristics that are different from the peripheral ones in glycolysis: higher activities as compared with the hepatic ones, and more sensitive to some neuronal changes (Siesjo, 1978).

From this point of view, it is necessary to investigate basal activities of hexokinase and phosphofructokinase in the rat brain, and also necessary to study their activities in the segmental portions of the brain.

In the case of different effect is detected among 3 portions, or of faint effect is detected in only one portion, if the experiment is performed using the sample prepared from whole brain, these effect is not able to detected. Because the brain was dissected into 3 portions.

In this report, hexokinase and phosphofructokinase activity, were examined in the dissected three brain portions including cerebral cortex, brain stem and cerebellum, using the experimental barbital dependent rat acquired by the barbital-admixed food treatment.

RESULTS

Fig. 1, 2 and 3 show the changes of hexokinase activities in the cerebral cortex, brain stem and cerebellum, respectively. The basal activities in these three portions were as follows (nmoles/mg protein/min): 20.8±7.24 in the cerebral cortex (n=6),

![Cerebral Cortex Graph](image)

Fig. 1. Effect of barbital dosing and its withdrawal on the hexokinase activity in the cerebral cortex.

Each group is consisted of 6 rats. Data are represented in mean±S. E. M. The significant differences in comparison to the non-treated rats (0 day of barbital dosing), are represented in * (p<0.05) and ** (p<0.01), respectively.
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**BRAIN STEM**

![Graph showing hexokinase activity in the brain stem over dosing and withdrawal periods.](image)

**CEREBELLUM**

![Graph showing hexokinase activity in the cerebellum over dosing and withdrawal periods.](image)

Fig. 2. Effect of barbital dosing and its withdrawal on the hexokinase activity in the brain stem portion.
Explanations are represented in Fig. 1.

Fig. 3. Effect of barbital dosing and its withdrawal on the hexokinase activity in the cerebellum.
Explanations are represented in Fig. 1.

20.1±1.46 in the brain stem (n=6), and 20.1±1.73 in the cerebellum (n=6). Among these three portions, the same tendencies were recognized during barbital dosing and its withdrawal. By barbital dosing, the hexokinase activities in these three portions decreased to approximately half as compared with these basal activities: they rapidly decreased within 2 days of its dosing, after which they remained at the stational level. At 6 hr after its withdrawal, their activities recovered to the non-treated level, afterwards them showing slight increases.

Fig. 4, 5 and 6 show the changes of phosphofructokinase activities in the cerebral cortex, brain stem and cerebellum, respectively. The basal activities in these three portions were as follows (nmoles/mg protein/min): 17.0±1.33 in the cerebral cortex...
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Fig. 4. Effect of barbital dosing and its withdrawal on the phosphofructokinase activity in the cerebral cortex.
Explanations are represented in Fig. 1.

Fig. 5. Effect of barbital dosing and its withdrawal on the phosphofructokinase activity in brain stem portion.
Explanations are represented in Fig. 1.

Fig. 6. Effect of barbital dosing and its withdrawal on the phosphofructokinase activity in the cerebellum.
Explanations are represented in Fig. 1.
Effect of barbital on glycometabolizing enzyme

(n = 6), 16.2 ± 0.64 in the brain stem (n = 6), and 14.7 ± 0.35 in the cerebellum (n = 6). The phosphofructokinase activities in these three portions changed in the same way as those of hexokinase. By barbital dosing, the phosphofructokinase activities decreased to approximately half in comparison with their basal activities: they rapidly decreased within 2 days of its dosing, after which they remained at the stationary level. At 6 hr after its withdrawal, their activities recovered to the non-treated level, afterwards them showing slight increases.

DISCUSSION

Both hexokinase and phosphofructokinase activities were depressed by the barbital dosing, the activity did not recovered to the control level at the later stage of the barbital dosing. The activities of these enzymes were increased after its withdrawal.

As to the inhibitory effects of barbital to these enzymes dosing, the hexokinase activity was found to be depressed more than the phosphofructokinase activity. On the contrary, the increased activity at 6 hr from barbital withdrawal, was larger in phosphofructokinase than in hexokinase. This difference between these two enzymes were considered to be due to the difference of participation in the "futile cycle" and in the allosteric effector. (Ui, 1977)

Among changes in three portions examined, almost same tendency in activity are detected. However, increases in the activity at early period of withdrawal, slightly deferent effect was recognized but it is not significant.

In the long term of barbital dosing and its withdrawal, as reported previously (Yanaura et al. 1983), 1) concentrations of glucose and glucose-6-phosphate were decreased at the 2 to 16 day of barbital dosing, while the concentration of glycogen was increased, 2) such changes, represented in 1), were returned to the control level at the later stage of barbital dosing, with them being considered to be due to the acquisition of biochemical tolerance, 3) the concentrations of glucose, glucose-6-phosphate and glycogen were decreased later than 24 hr from barbital withdrawal, 4) the concentration of lactate showed no change during the barbital dosing, while it was increased after barbital withdrawal. As mentioned above, the activities of these two rate-limiting enzymes in the cerebral glycolysis system remained depressed throughout barbital dosing, and showed opposite changes in relation to the changes of concentrations of glycolytic intermediates. Such differences between these enzyme activities and the concentrations of glycolytic intermediates, suggested that the glucose metabolism system was mediated by many mediating factors, and considered to be caused by the changes in balance of mediating effectors. In addition, the metabolic intermediates in the glycolysis were thought to be better markers, in comparison with the glycolytic enzymes, for the differentiation of acquisition of dependence and tolerance in barbital treatment.

It was observed that the activities of rate-limiting enzymes in glycolysis, increased rapidly after barbital withdrawal, after rapid increases they increased gradually. In
such biphagic changes, the first rapid increases could be conside to be a measure preceding the appearance of the withdrawal symptomes, in order to investigate the mechanism of the withdrawal. In addition, from the phenomenon that the activities of rate-limiting enzymes started increasing at 3 to 6 hr after the withdrawal, it is considered that some biochemical sequences start to guard against the biochemical changes, namely the withdrawal syndrome, which was induced by the withdrawal.

REFERENCES


