BIOCHEMICAL ASPECTS OF EXPERIMENTAL BARBITAL DEPENDENCE II: EFFECT ON GLYCOMETABOLISM

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Abstract—A single injection of barbital increased glycogen, while it decreased glucose and glucose-6-phosphate levels in the rat brain. In long barbital dosing (36 days), however, the metabolite level of carbohydrate was almost recovered to the non-treated level. At the later stage of withdrawal (24–48 hr), all metabolites examined except lactate decreased. Only lactate increased remarkably. The effect of barbital dosing and withdrawal was almost same in the three portions, i.e., the cerebral cortex, brain stem, and cerebellum. Barbital depresses the central glycometabolism, and at the dependent stage (long term barbital dosing, 36 days or more), metabolism was almost same as the control. At the later period of withdrawal, it appeared that lactate was increased because of the hypoxic condition caused by stroke. In conclusion, carbohydrate metabolism can probably serve as a sensitive measure for the development of barbital dependence and the onset of withdrawal.

Neurochemical aspects have been an important measure to define the magnitude of barbital dependence and withdrawal. However, few reports have been available. Cerebral biochemical changes perhaps have an important role in the occurrence of barbital dependence and onset of withdrawal syndrome.

In order to clarify the relationship between neurochemical changes and barbital dependence, it may be important to obtain evidence about neurochemical changes induced by barbital intoxication. Changes in activity of monoaminergic neurons (1–3) and the cyclic-adenosine-3',5'-monophosphate system had been observed. Especially, carbohydrate metabolism in the nervous system is one of the important markers of neuronal activity. Hypoglycemia is able to induce convulsion (4), and the main behavioral change induced by barbital withdrawal is convulsion; from this standpoint, carbohydrate metabolism may be influenced by barbital intoxication. On epileptic seizure, rate of glycolysis and hexokinase activity are depressed (5). The onset of withdrawal may have a similar influence. In addition, carbohydrate metabolism in the nervous system was sensitively influenced by various factors such as neural activity (6–8) and neurotropic drug dosing (9, 10).

Morgan et al. (1–3) previously reported that the turnover rate of norepinephrine and dopamine was influenced by barbital intoxication, however, changes in carbohydrate metabolism in barbital dependence and withdrawal have not yet been examined.

In this report, the effect of barbital dosing and withdrawal on the glycometabolizing system was examined using experimental dependent rats obtained by barbital-admixed food treatment.
Materials and Methods

Animals: Male Sprague Dawley rats were used for examination. Barbital was dosed according to the schedule shown in Fig. 1. Animals were sacrificed for preparation of samples at the 16th day of barbital dosing (this period may be a mild state assessed from behavioural changes as the result of withdrawal); at the 36th day of barbital dosing (at this period, remarkable behavioural changes were induced by withdrawal, i.e., convulsion); and the 17th, 24th, 48th hr of withdrawal.

Pentylenetetrazole (80 mg/kg) was injected subcutaneously to non-treated rats as a convulsion inducing agent and were sacrificed upon clonic-tonic convulsion. Acute barbital was examined by injection of barbital (200 mg/kg) intraperitoneally 45 min before sacrifice. Each experimental group consisted of 6 animals.

Biochemical procedure: The brain was removed and dissected into 3 portions, i.e., the cerebral cortex, brain stem and cerebellum. In all procedures before deprotenization, the tissue was handled under conditions of cooling by liquid nitrogen. Each frozen tissue was homogenized by 4 volumes of 0.4 N perchloric acid using a Polytron (Kinematics Co., Switzerland). The supernatant was used for enzymatic assay.

Assays of each component, glycolysis (glucose, glucose-6-phosphate and lactate) and glycogen were performed by enzymatic methods using NAD (NADH) or NADP (NADPH) coupled reactions as previously reported (11). These biochemical measurements were performed in duplicate. Tissue recovery was 98.5% and concentration in this report was corrected by this recovery.

Drugs used: Pentylenetetrazole was obtained from the Sigma Co., and other biochemical reagents were obtained from Boehringer Mannheim, GmBH. Barbital and barbital sodium was obtained from the Wako Chemical Co.

Results

Effect of pentylenetetrazole-induced convulsion on the concentration of glucose, glucose-6-phosphate, glycogen, and lactate in the cerebral cortex, brain stem, and cerebellum are shown in Table 1. Pentylenetetrazole was used as the inducing agent for convulsion. The glucose concentration was significantly decreased in both the cerebral cortex and brain stem. At the 36th day of barbital dosing (at the dependent stage), however, the concentration recovered to the same level as the control group (3.930±0.345, N.S.). At seventeen, 24 and 48 hr of withdrawal, the concentration decreased again (Fig. 2-a).

In the brain stem portion (Fig. 2-b), glucose changed in the same manner as in the cerebral cortex. The basal level of glucose was 4.230±0.313. In the cerebellum (Fig.
### Table 1. Effect of pentylenetetrazole induced seizure on central carbohydrate metabolism

|                | Cerebral cortex | Brain stem | Cerebellum |
|----------------|----------------|------------|------------|
|                | Control        | PTZ        | Control    | PTZ        | Control    | PTZ        |
| Glucose        | 4.0617±0.2923  | 0.8150±0.1619** | 4.2250±0.3125 | 2.2333±0.2011** | 2.9083±0.4063 | 2.2117±0.3202 |
| Glucose-6 phosphate | 0.1037±0.0114  | 0.1400±0.0145   | 0.1287±0.0145 | 0.1728±0.0180 | 0.1473±0.0146 | 0.1342±0.0199 |
| Glycogen       | 1.5817±0.1231  | 1.6167±0.1421   | 1.9217±0.0862 | 1.6750±0.4659 | 1.7550±0.2142 | 1.3917±0.0704 |
| Lactate        | 1.7212±0.4251  | 1.6167±0.3130   | 1.8417±0.2241 | 1.3317±0.1780 | 1.2433±0.1628 | 1.7817±0.4603 |

The data are the mean±S.E.M. of 6 rats. Each assay was performed in duplicate. The unit of concentrations are nmoles/mg wet weight. Sample solution of glycogen prepared from 1 mg wet weight tissue by amiloglycosidase. **P<0.01, compared with the non-treated group.

### Table 2. Effect of barbital dosing and withdrawal on neural glycogen concentration

|                | Cerebral cortex | Brain stem | Cerebellum |
|----------------|----------------|------------|------------|
| Control        | 1.5817±0.1731  | 1.9217±0.0862 | 1.7550±0.2142 |
| Barbital injection | 3.9450±0.3241** | 2.6433±0.1883** | 2.2683±0.2413 |
| 16th day of dosing (mild state) | 4.6220±0.5359** | 2.5700±0.2986** | 1.8020±0.3797 |
| 36th day of dosing (sever state) | 1.6517±0.1198  | 1.8717±0.3628  | 1.5417±0.1725 |
| 17hr of withdrawal | 1.2800±0.2630  | 1.5050±0.2065  | 1.5217±0.3611 |
| 24hr of withdrawal | 0.8617±0.0616** | 0.7333±0.1166** | 0.8317±0.1966** |
| 48hr of withdrawal | 0.6617±0.1360  | 0.6900±0.1783** | 0.5750±0.0936** |

**P<0.01, compared with the non-treated group.
Fig. 2. Effect of barbital dosing and withdrawal on glucose concentration. Each data point is the mean ±S.E.M. of 6 rats.

The effect of barbital injection and dosing and withdrawal on the cerebral glucose-6-phosphate level are shown in Fig. 3-a. At the 16th day of barbital dosing, barbital depressed the glucose-6-phosphate concentration. At the 36th day of barbital dosing (dependent stage), however, the concentration recovered to the control level. At the 17th hr of withdrawal, the concentration was slightly increased over that of the control level. At the later stage of withdrawal, the level decreased. In the brain stem portion, barbital injection decreased the concentration of glucose-6-phosphate. However, at the dependent stage, the level recovered to the control level. At the early stage of withdrawal, the level was not affected. At the later period of withdrawal, the concentration decreased. These biochemical changes were almost the same as those in the cerebral cortex (Fig. 3-b). In the cerebellum, the effect of barbital was similar to those in other brain portions.

Barbital dosing did not affect the lactate concentration in the cerebral cortex, and at the early period of withdrawal, almost the same concentration was detected. However,
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Fig. 4. Effect of barbital dosing and withdrawal on lactate concentration.

at the later period (24–48 hr) of withdrawal, the concentration was significantly increased (P<0.01) as compared to the control. (Fig. 4-a). At the later stage of withdrawal, the rats had strokes. In the cerebellum (Fig. 4-c), almost the same effect of barbital was recognized. In the brain stem portion (Fig. 4-b), on the 36th day of barbital dosing, however the lactate began to increase; long barbital dosing most severely affects the brain stem in a hypoxic condition.

The effect of barbital dosing and withdrawal on glycogen concentration are shown in Table 2. The basal level of cerebral glycogen was 1.5817±0.1232 nmoles glucose/mg wet weight. Barbital injection increased the concentration (3.945±0.3241, P<0.01); and at the 16th day of barbital dosing (mild stage), the concentration was also higher than the control level (4.622±0.5359, P<0.01). At the 36th day of barbital dosing (severe stage), dependence completely developed. The glycogen level recovered to the control level. At 17 hr of withdrawal, the level was not different from the control. At the later stage (24-48 hr) of withdrawal, the glycogen concentration decreased significantly. The changes in glycogen concentration in the brain stem portion were the same as those of the cerebral cortex. Barbital had an increasing effect on the glycogen concentration during the dosing period; however, the glycogen level was significantly decreased at the later stage of withdrawal. In the 3 portions examined, i.e., the cerebral cortex, brain stem, and cerebellum, almost the same effects of barbital dosing and withdrawal were recognized.

Discussion

Central carbohydrate metabolism is a sensitive marker of neural activity (12). For example, electroshock seizure affects neural carbohydrate metabolism (7, 8), neurotropic drug dosing modulates glucose metabolism (9, 10, 13), ischemia depresses carbohydrate metabolic activity (6), and exposure to atmospheres containing high concentration of CO₂ (14) changes the cerebral glycometabolism.

Generally, these changes in neural glycometabolism were mediated by some neural change, e.g., monoamine system and the cyclic nucleotide system. Barbital dosing affects the monoaminergic neuron or cyclic nucleotide system, and changes in neural carbohydrate metabolism will follow. Morgan et al. (1–3) previously observed the change in the norepinephrine system. These changes related to dependence or withdrawal may affect neural carbohydrate metabolism. Carbohydrate metabolism was strongly
regulated by monoamines, cyclic nucleotides, and some proteohormones. Because of this complicated regulation system, carbohydrate metabolisms are sensitive to various neurochemical changes; therefore, carbohydrate metabolism can possibly be considered as a marker for dependence and withdrawal.

Barbital dosing affects the carbohydrate metabolism in a complicated manner. Perhaps, these changes that occur depend on the specificity of neural and glial glycometabolism in the brain, i.e., 1) very low concentration of glycogen (1.5–2.0 μmoles), 2) high activities of neural hexokinase activity (20-fold higher than the liver) and other kinases (phosphofructokinase, phosphoglycerate kinase activity, pyruvate kinase activity, 3) hexokinase and phosphofructose kinase are rate determining enzymes and very complicated 'metabolic inhibition' was observed. The main factors that regulate the glycolytic pathway and the TCA cycle are the consumption and supply of energy (i.e., ATP and creatine phosphate).

Hypothermia or stress decreases the turnover rate of creatine phosphate, adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, and inorganic phosphate (15). Activation of neural activity generally caused decrease of adenosine, and glycolysis and the citrate cycle are facilitated. Hypothermia induced by barbital and barbital dosing for 2 weeks are regarded as the stressful stage for rats. Cerebral carbohydrate metabolism was affected the most. A long term dosing of barbital (for 36 days), however, evidently has little effect on glycometabolism. At the 36th day of barbital dosing (severe state), the adenylate cyclase activity also recovered to the control level (Fig. 5).

The glycometabolism was in accordance with our previous report about the adenylate cyclase activity. At 17 hr of withdrawal, adenylate cyclase activity increased, and the glucose level decreased. At this stage, both the creatine phosphate level and adenosine triphosphate level increased more than those of the control (16). The enzyme activity of the glycolytic pathway during this period were evidently activated more than those at the dependent state. Further examinations on the carbohydrate metabolisms during this early period of barbital withdrawal (within 24 hr) must be carried out.

Furthermore, barbital affects the GABAnergic system (17–19), and the GABA shunt is related to the TCA cycle. The convolution was also related to the GABAnergic system: at seizure, GABA was decreased, and the GABA-glutamate-glutamine equilibrium was changed (19, 20). The withdrawal syndrome was caused by GABAnergic charge, and barbital has GABA-like action (22, 25). These complicated relations may influence glucose and glucose-6-phosphate concentrations. As the result of the changes of glucose and glucose-6-phosphate, glycogen was accumulated at the dependence developing stage, and it was reduced to the control level at early period of the withdrawal stage. At 24 to 48 hr of withdrawal, stroke was recognized, and the reduction of cerebral blood flow ensues. At this period, the lactate...
level significantly increased; generally, lactate is an anaerobic metabolite of glycolysis. Anoxia caused by stroke and change of oxygen supply caused by convulsion enhance the lactate production as the result of inhibition of the TCA cycle.

In conclusion, 1) barbital depresses the neuronal activity that caused depression in the glycolytic pathway, 2) at the late stage of the withdrawal period, the anaerobic condition was recognized, 3) equilibrium of the glycolytic pathway may become the sensitive measure for barbital dependence.

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