METHODS

Immunochemical Assay of Glia-Specific Antigens as a Criterion for Blood-Brain Barrier Permeability in Rats during Acute Intoxication with Sodium Barbital

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Enzyme immunoassay showed penetration of two glia-specific antigens, glial fibrillar acid protein GFAP and specific brain glycoprotein α_2GP, through the blood-brain barrier in rats treated with toxic doses of sodium barbital. The permeability of the blood-brain barrier was completely normalized 3 days after treatment. This method can be used in clinical practice for evaluation of the severity of impairment and dynamics of normalization of blood-brain barrier properties during acute intoxication with barbiturates.

Key Words: *blood-brain barrier; glia-specific antigens; GFAP and α_2GP; sodium barbital*

Overdosage of psychotropic and hypnotic drugs is the most typical cause of drug intoxication. Barbiturates belonging to hypnotic drugs can produce toxic effects on the central nervous system (CNS) and suppress its activity [1,10].

Most reports concerning the effects of barbiturates on the permeability of the blood-brain barrier (BBB) were focused on determination of blood-brain distribution constants for some circulating substances [5] and molecular mechanisms underlying transport of low-molecular-weight substances (sugars, amino acids, *etc*.) through endotheliocyte membranes in brain capillaries [7]. However, there are no data on BBB permeability for low- and high-molecular-weight substances from the brain into the blood during barbiturate treatment.

Nervous tissue-specific marker proteins are of particular interest in this context. These proteins are present in the plasma in threshold concentrations under normal conditions, but their content sharply increases after exposure to damaging factors enhancing BBB permeability [2,3,8,11].

Here we evaluated the possibility of using enzyme immunoassay of glia-specific antigens (GSA) for evaluation of BBB permeability during acute intoxication with sodium barbital.

MATERIALS AND METHODS

Experiments were performed on 130 outbred male albino rats weighing 200±20 g. The rats were kept under natural light-dark cycle and free access to water and food.

Acute intoxication with sodium barbital was modeled as described elsewhere [1]. Sodium barbital (10% water solution) was injected intraperitoneally. The rats awoke 560±38 min after injection of 280 mg/kg sodium barbital (narcotic dose) died from apnea 163±18 min after injection of sodium barbital (lethal dose).

Thirty rats injected with an equivalent volume of distilled water served as the control. The blood was

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taken 1, 2, 6, 12, 24, 72, and 144 h after injection of sodium barbital in a narcotic dose (10 rats per point). In rats receiving a lethal dose (n=30), the blood was taken 1 and 2 h after treatment or after death (10 rats per point).

The blood was taken from the caudal vein. The serum was obtained routinely and stored at -18°C.

Astrocyte cytoplasmic antigens, glial fibrillary acidic protein (GFAP, molecular weight 53±5 kDa) [4] and brain-specific glycoprotein α_2GP (molecular weight 50±5 kDa) [13], were used as markers of BBB permeability (from the brain to the blood). Since these rat proteins immunochemically cross-react with the corresponding human molecules and have similar physicochemical, molecular, and biological properties, we can extrapolate experimental results to processes in the human body.

GSA concentration was measured by sandwich enzyme immunoassay [12]. Monospecific antisera, antibodies against GFAP and α_2GP, and corresponding conjugates were obtained and GFAP and α_2GP concentrations were calculated as described elsewhere [2]. The results were analyzed by Student's t test. The differences were significant at $p<0.05$.

RESULTS

Enzyme immunoassay kits for GSA reliably and reproducibly detect GFAP and α_2GP in concentrations of 1-128 and 0.9-102 ng/ml, respectively (Fig. 1).

In control rats, the maximum plasma concentrations GFAP and α_2GP did not exceed 16 and 15.6 mg/ml, respectively (means 7.6±1.2 and 8.0±1.3 ng/ml, respectively). These values were taken as normal plasma contents of rat GFAP and α_2GP.

In rats receiving sodium barbital in a narcotic dose, the concentrations of GFAP and α_2GP increased 1 h postinjection (to 44.8±2.3 and 53.1±1.9 ng/ml, respectively, Fig. 2), remained practically unchanged from the 2nd to 12th hour (69.7±3.1 and 69.7±0.8 ng/ml, respectively), decreased 24 h postinjection (to 33.77±2.02 and 38.13±2.10 ng/ml, respectively), and did not differ from normal after 72 hours.

In rats receiving sodium barbital in a lethal dose, the concentrations of GFAP and α_2GP 1 h postinjection increased to 96.0±3.2 and 83.6±2.3 ng/ml, respectively, and remained unchanged until death (3 h postinjection, Fig. 3).

The increase in plasma GFAP and α_2GP concentrations in rats receiving sodium barbital in a lethal dose significantly ($p\leq0.05$ and $p<0.002$, respectively) differed from that in animals receiving a narcotic dose (1 and 2 h after treatment). A 2-fold increase in the dose of sodium barbital promoted penetration of glia-specific marker proteins through BBB into the blood,

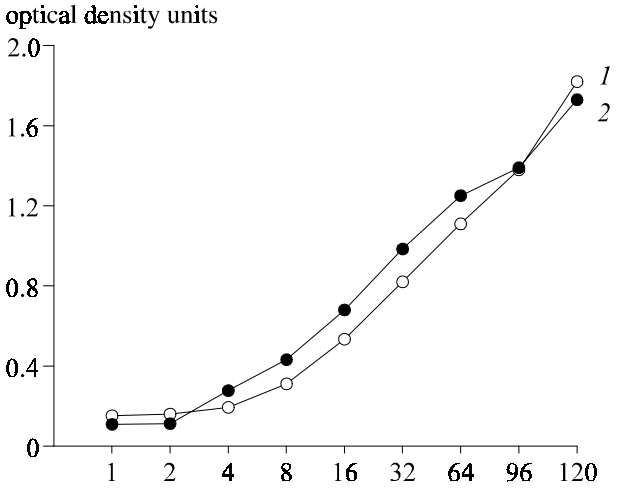

Fig. 1. Calibration curves of GFAP (*1*) and α_2GP (*2*) enzyme immunoassay.

i.e., changes in BBB permeability correlated with the dose of sodium barbital. Therefore, sodium barbital in a toxic dose changed BBB permeability for glia-specific proteins GFAP and α_2GP. Disturbances in BBB permeability caused by sodium barbital in a narcotic dose persisted for 72 h.

Plasma GFAP and α_2GP concentrations at the very early stages of acute intoxication (2 h postinjection) 6-9-fold surpassed the control, which indicated severe damage to cell membranes forming BBB. The content of these proteins returned to normal 3 days after treatment, which indirectly indicated recovery of BBB functions.

It was reported that barbiturates in low doses stabilize BBB permeability [9]. Our results agree with the hypothesis of M. Gumerlock, *et al*. [6]. According to this hypothesis, this effect of barbiturates is related to

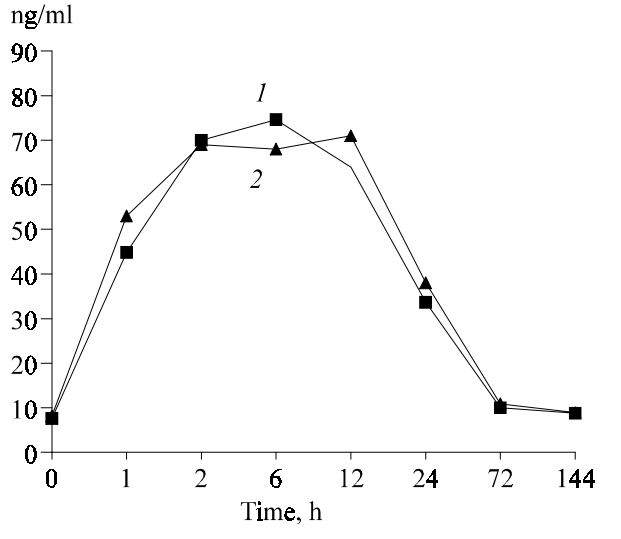

Fig. 2. Dynamics of GFAP (*1*) and α_2GP (*2*) concentrations after injection of sodium barbital in a narcotic dose.

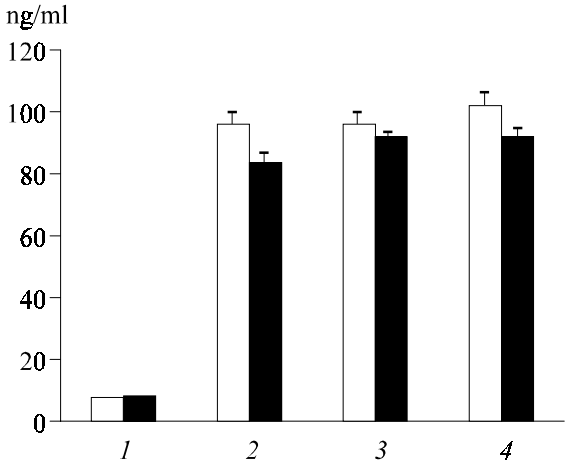

Fig. 3. Dynamics of GFAP (light bars) and α_2GP (dark bars) concentrations after injection of sodium barbital in a lethal dose: control (*1*), 1 (*2*) and 2 h after treatment (*3*), and postmortem (*4*).

their hypotensive action, rather than to their direct influence on BBB components. By contrast, barbiturates in toxic doses directly affecting cytochrome P-450 metabolism and enzyme systems involved in glycolysis, oxidative phosphorylation, and transmembra-ne transpor can modulate morphofunctional organization of BBB components, change its permeability, which can be evaluated by plasma GSA concentrations.

Our findings suggest that immunochemical analysis of GSA can be used in clinical practice for evaluation of the severity of functional changes in BBB during acute intoxication with barbiturates.

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