Permeability of the Blood-Brain Barrier in Newborn Rats with Induced Hyperthermic Convulsions

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The permeability of the blood-brain barrier in the brain-blood direction is examined in newborn rats with hyperthermic convulsions induced by exposure to infrared radiation. Impaired permeability of this barrier due to convulsions is characterized by increased serum concentration of glial fibrillary acidic protein. Dexason lowers the protein level, which confirms its stabilizing effect on the blood-brain barrier structure. The advantage of early dexason administration is shown.

Key Words: *blood-brain barrier; glial fibrillary acidic protein; dexason; infrared radiation; enzyme-linked immunosorbent assay*

The effect of hyperthermia on the central nervous system (CNS) and on the function on the blood-brain barrier (BBB) has been extensively studied [3-5,7]. Impaired resistance and destruction of the BBB in the blood-brain direction have been described in detail in animal models of hyperthermic convulsions induced by electromagnetic microwave radiation acting on the CNS [4,6].

The permeability of the BBB in the reverse direction, i.e., brain-blood, has been poorly investigated, although analysis of its mechanisms may, on the one hand, help understand the pathogenesis of adverse effects produced by high temperatures on the CNS and, on the other hand, be of applied importance for evaluating the degree of damage to the BBB and monitoring the efficacy of therapy.

The aims of the present study were to examine the permeability of the BBB in neonatal rats with induced hyperthermic convulsions and to assess the stabilizing effect of dexason (DS) on the BBB function.

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MATERIALS AND METHODS

Destruction of the BBB was achieved by inducing convulsive paroxysms with infrared (IR) radiation as described J. A. McCaugran *et al.* [2]. The source of IR radiation was an incandescent lamp of 200 W located 25 cm above the surface being irradiated. Pups were irradiated in a wooden box (13×13×20 cm) with holes having a total area of 38 cm^2 in its side walls.

A total of 492 random-bred newborn rats (aged 5-8 days) of both sexes weighing 12.4±3.8 g were used. The animals were divided into five groups: rats injected with the glucocorticoid DS 3 h before radiation exposure (group 1, n=109); rats irradiated without DS pretreatment (group 2, n=193); rats injected with DS 30 min after convulsions (group 3, n=24); rats injected with DS 90 min after convulsions (group 4, n=13); and rats injected with DS 3 h after convulsions (group 5, n=12). The first control group consisted of 69 healthy newborn pups. To assess the effect of DS on the BBB function in healthy newborn rats, a second control group (n=42) was used, which received DS without being irradiated; DS was injected intraperitoneally in a dose of 5 mg/kg 3 h

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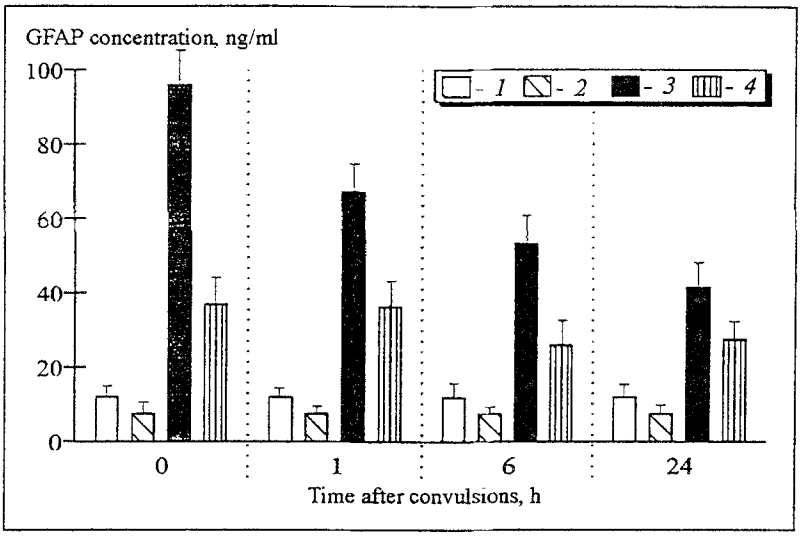

Fig. 1. GFAP concentration at different times after induced convulsions. Here and in Fig. 2: *1*) control; *2*) control+dexason; *3*) convulsions; *4*) convulsions+ dexason.

before blood collection. A third control group (n=30) received placebo (water for injection intraperitoneally in a dose of 100 μl), and their blood was collected 5.5, 4.5, and 3 h later.

Rats of test groups 1 and 2 were exposed to four and five radiation sessions, respectively, while those of test groups 3, 4, and 4 were exposed to one session.

In test groups 1, 3, 4, and 5, DS (KRKA) was injected intraperitoneally in a dose of 5 mg/kg.

Clinical picture of convulsions, differences in clinical manifestations between the groups exposed to radiation once and repeatedly, and the dependence of convulsive paroxysms on the preirradiation injection of DS were analyzed. In addition, the time between the start of radiation and the appearance of convulsions was recorded, and serum level of glial fibrillary acidic protein (GFAP) was measured.

Blood was collected by decapitation. It was taken at the height of convulsions and 1, 6, and 24 h after convulsions in groups 1 and 2; 6 and 72 h after irradiation in test group 3; and 6 h after irradiation in groups 4 and 5.

Serum GFAP was measured using sandwich ELISA [8] in our modification [1]. Kits for the determination of GFAP standardized for accuracy, reproducibility, and reliability in the GFAP concentration range 1-128 ng/ml were developed at the Laboratory of Immunochemistry of the V. P. Serbskii State Research Center of Social and Forensic Psychiatry, Moscow.

RESULTS

Clinical picture of induced hyperthermic convulsions in newborn rats. IR radiation from a 200 W incandescent lamp located 25 cm from a newborn rat caused the following changes in the behavior and general condition.

Phase 1: a short (about 30 sec) period of marked general hypodynamia after several seconds of exposure to IR radiation, followed by motor agitation characterized by "swimming" movements of the fore and then hind limbs. Subsequently, motor agitation progressed, the tail became rigid, and the skin turned bright pink with the development of acrocyanosis gradually transforming into total cyanosis.

Phase 2: tonic convulsions with pronounced opisthotonos, accompanied by loud squeaking and urination. Some rats showed ataxia and sporadic myoclonic twitching of the hind limbs. Rats that developed a convulsive status with opisthotonos were taken away from the exposure area.

Phase 3: a state similar to catatonia. During this period rats responded to tactile and auditory stimulation by serial long-lasting clonic-tonic convulsions followed by prolonged hypokinesia. The cyanotic skin became pale. Motor and reflex activities were fully restored in 4-6 h.

The time-course of the clinical picture of a convulsive attack after the first exposure to IR radiation was similar in all groups. In the group preinjected with DS, the clinical picture described above developed later, convulsive attacks and the catatonia phase were much shorter, and the recovery of motor and reflex activities occurred much earlier.

After the second irradiation session, the period of general hypodynamia was longer than after the first. Subsequently, enhanced motor activity with hyperkinesia of the fore and hind limbs was observed for a short time and was suddenly succeeded by a phase of tonic convulsions. As during the first irradiation session, rats assumed an opisthotonoid posture, and pronounced convulsions were accompanied by loud squeaking and urination. All rats showed ataxia and sporadic myoclonic twitches. The

phase of catatonia was longer. Reflexes and motor activity were restored only after 8 h. In the group given DS, the severity of convulsive attacks, as assessed by the average duration of convulsions, was virtually the same as in rats which were not injected with the glucocorticoid before irradiation.

ELISA analysis of serum GFAP in normal rats and rats with induced hyperthermic convulsions. The ELISA test system for analyzing GFAP was used to assess BBB permeability in the brain-blood direction. The mean serum GFAP level in control group 1 was significantly higher than that in control group 2 which received DS (12.0±1.4 ng/ml and 7.4±1.1 ng/ml, respectively; $p<0.02$). The mean GFAP concentration in the placebo control did not differ from that in control group 1.

We also assessed BBB permeability at different times after the first series of radiation-induced convulsions (Fig. 1). It should be noted that the phenomenon of GFAP entry into the bloodstream, which indicates impaired BBB permeability, was observed in control and test groups throughout the observation period, although serum GFAP levels in experimental animals were significantly higher than in the control. ELISA at different intervals after irradiation showed higher serum GFAP concentrations in the rats given DS before convulsions (mean concentration 96 ng/ml) than in those pretreated with DS (36 ng/ml).

A progressive decline in GFAP levels was observed in experimental animals: 1 h after the induced convulsions the GFAP level was 36.3 ng/ml in the DS-pretreated rats and 67.2 ng/ml in rats that did not receive DS ($p<0.02$); the GFAP levels in these groups were 26 and 53.3 ng/ml, respectively, 6 h after convulsions ($p<0.02$) and 27.4 and 41.6 ng/ml at 24 h ($p<0.05$).

The optimal time of DS administration to newborn rats after the IR radiation-induced impairment of BBB permeability was determined. Administration of DS 30 min postirradiation reduced BBB permeability ($p<0.05$) compared with that observed in the absence of DS (Fig. 2). DS injection 1.5 or 3 h postirradiation reduced BBB permeability to a lesser extent, the mean GFAP levels being 40.6 and 61.3 ng/ml, respectively (Fig. 2).

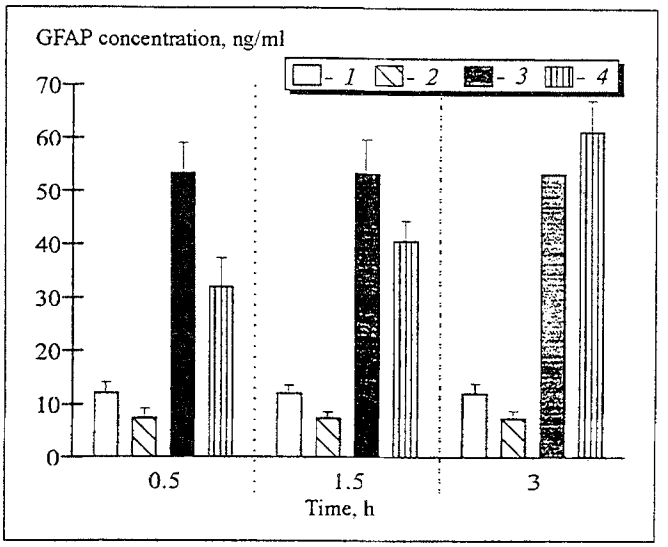

Fig. 2. GFAP concentration at different times after dexason injection.

Thus, we have demonstrated impairment of BBB permeability in newborn rats with IR radiation-induced hyperthermic convulsions characterized by increased GFAP entry into the blood. The glucocorticoid DS exerted a stabilizing effect on the BBB structure manifesting itself as a statistically significant reduction in the degree of GFAP elimination into the blood. The advantage of early DS administration was shown.

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