EFFECT OF DESFERRIOXAMINE (DESFERAL) ON EPILEPTIC FOCUS FORMATION IN RATS AFTER SUBDURAL INJECTION OF BLOOD

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One of the most serious complications of craniocerebral trauma (CCT) is the development of seizures in the subacute and late periods after trauma [4, 10]. It has been shown that subpial conjections of iron-containing compounds of blood and iron salts into a region of the cortex induce the formation of a chronic epileptic focus in experimental animals [8, 11, 12]: epileptic discharges arise 4-10 days after the injection procedure [8, 12]. The mechanisms of the epileptogenic action of iron have not yet been fully studied. However, we know that an essential role in the regulation of lipid peroxidation (LPO) activity of which is accompanied by the development of seizures [2, 3], and in the generation of free radicals in biological systems, is played by the oxidation—reduction pair Fe^{2+} and Fe^{3+} [1].

This paper describes a study of the action of desferrioxamine (DF), a chelating agent of free trivalent iron — on the development of epileptic activity in rats after subdural injection of blood into the region of the sensomotor cortex.

METHODS

Experiments were carried out on 80 mature male Wistar rats weighing 200-250 g. The animals were kept under identical conditions, including an identical diet and water supply. The anesthetized animals (pentobarbital sodium, 40 mg/kg) were fixed in a stereotaxic apparatus, where 5 μliters of freshly collected whole blood, obtained from the same animal, was injected through the dura mater through burr holes to a depth of 1.5 mm by means of a microsyringe, into the region of the sensomotor cortex of the left hemisphere. The burr hole was then closed with a plug consisting of quick-hardening acrylic plastic and the wound was closed with silk thread sutures. During the operation, in 20 rats, nichrome electrodes insulated with fluorine plastic, and with an area of active surface of 2 mm^2 were applied to the surface of the skull in order to record the EEG. The reference electrode was located in the occipital region of the head, subcutaneously. The animals were divided into two groups: control (group 1) and experimental (group 2). The experimental group of rats was given an intramuscular injection of 0.4 ml of an aqueous solution of DF in a dose of 80 mg/kg, whereas animals of the control group received an injection of 0.9% sodium chloride solution in the same volume. DF was injected from the 1st day after the operation daily for 30 days. The EEG was recorded 2, 4, 6, 8, 10, 15, 20, 25, and 30 days after the operation. The other 60 rats had an EEG record of the effect of myorelaxants on excited conditions in 2, 10, 15, 20, 25, and 30 days after the operation. For this purpose the rats were given an intramuscular injection of 4 mg/kg of d-tubocurarine, and 0.5% procaine solution was injected into the scalp, after which the head was gently fixed. The rats were maintained on artificial respiration through the nasal passage by means of a special apparatus. After division of the soft tissues of the skull nichrome electrodes were introduced into the region of the burr hole, and the reference electrode was applied to the surface of the head in the occipital region. The EEG was recorded by means of a Medicor electroencephalograph (Hungary), and subsequently recorded on a Tesla tape recorder (Czechoslovakia), and also from the output of the electroencephalograph on paper tape. The EEG was processed by a signals analyzer (Brüel and Kjaer, Denmark).

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RESULTS

The main criterion for assessing the formation of a chronic epileptic focus was the frequency of epileptic discharges in the EEG. The number of spikes and the number of spike—slow wave complexes throughout the period of recording was analyzed and subsequently expressed as the number of discharges per minute. The duration of each recording in animals with chronically implanted electrodes was 30 min, whereas in animals taking part in the acute experiments it was 60 min. Epileptic activity of animals of the control group (1) was exhibited from the 2nd day after the operation in the form of spikes with an average frequency of 3.78 ± 0.68 min and of spike—slow wave complexes with a frequency of 4.25 ± 0.85/min. Both single spikes and long discharges of epileptic activity were recorded in all the animals. No spike—slow wave complexes were observed in three of the 30 animals on the 2nd, 15th, and 25th days after the operation, under acute experimental conditions. With an increase in the time after the operation an increase in the number of epileptic discharges was observed to reach a maximum by the 8th day; the frequency of the spikes was 42.9 ± 13.4/min and of spike—slow wave complexes 4.8 ± 0.96/min. Later, no significant changes of discharge frequency relative to this level were observed. On the 2nd day after the operation the frequency of epileptic discharges in animals of the experimental group (2) was comparable with the discharge frequency in the control group (1). On the 4th day after the operation a significant decrease in the number of epileptic patterns was observed compared with the control group: the frequency of spikes in group 1 was 22.28 ± 6.9, and of group 2 — 5.82 ± 1.23/min ($p < 0.01$); the frequency of spike—slow wave complexes was 6.28 ± 1.57 and 0.26 ± 0.02/min in groups 1 and 2, respectively ($p < 0.01$). Thereafter and until the 10th day, spike—slow wave complexes disappeared completely, and the frequency of spikes was 2.85 ± 0.6/min ($p < 0.001$). On the 30th day after the operation infrequent single spikes with a frequency of 0.24 ± 0.001/min were recorded in the EEG. A characteristic feature of the electrical activity of the brain in animals receiving DF was a decrease in amplitude of the EEG waves and an increase in frequency of the biopotentials.

Much has been published on the involvement of LPO in the mechanisms of epileptogenesis [2, 3]. The most important role in the regulation of LPO processes is played by iron ions [1]. Addition of iron ions or blood-containing compounds to a cell suspension or to solutions containing unsaturated fatty acids induce the generation of free oxygen radicals and peroxides [4, 5]. During autooxidation of oxyhemoglobin and methemoglobin, superoxide radicals also are generated. Interaction of free oxygen radicals and peroxides with components of cell membranes induces degenerative changes, associated with polymerization of protein chains and destruction of sulfhydryl groups [6, 9]. Injection of blood and hemoglobin into the subarachnoid space of animals causes morphological changes similar to those observed in post-traumatic epilepsy in man. It was shown previously that DF reduces the content of low-molecular-weight chelated iron and inhibits growth of malonic dialdehyde in the dog's brain after cardiac arrest and subsequent resuscitation of the animals [7]. Injection of DF, which binds trivalent iron ions (not entering into ferritin), into rats caused prevention of the formation of a chronic epileptic focus after subdural injection of blood, which was shown by gradual reduction of the epileptic discharges and their complete disappearance by the 30th day after the operation. In animals not receiving DF, progressive development of epileptic activity was observed in the region of injury throughout the period of observation.

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