PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Effect of Superoxide Dismutase on Convulsive Seizures in Rat Models of Epilepsy

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Intravenous injections of superoxide dismutase into rats with amygdaline kindling mitigated spontaneous and electrostimulation-induced epileptic seizures and raised the thresholds of electric current for their elicitation; these seizures of reduced intensity were observed during 3 days following chronic (for 6 days) treatment with this enzyme. In contrast, superoxide dismutase injections had no effect on the convulsions induced by electric shock or corazole.

Key Words: *epileptic activity; amygdaline kindling; superoxide dismutase*

A number of pathological conditions including, among others, arterial hypertension, brain injuries, and tissue ischemia after heavy blood loss, involve functional and biochemical changes that lead to the formation of free radicals [3,7]. These radicals can probably be generated in the course of arachidonic acid metabolism [10,15], which is enhanced during convulsions [4]. It has also been shown that the intensified synthesis of cyclooxygenase products in the brain of animals with experimentally induced convulsive seizures may be accompanied by the production of free oxygen radicals [1,7].

Superoxide dismutase (SOD; EC 1.15.1.1) is an enzyme catalyzing the reaction of superoxide radical disproportionation and thereby reducing concentrations of this radical in tissues. Exogenous SOD injected into the amygdala has been shown capable of reducing the brain's convulsive activity resulting from kindling [9,11], but the impact of SOD on physiological parameters following its entry into the circulation has not been addressed. To gain better insight into the mechanism by which SOD exerts an anticonvulsive effect, it is important to establish whether SOD is able to act within the vascular bed, for, being a high molecular protein, this enzyme is likely to cross the blood-brain barrier only in minute quantities if at all.

In this work we examined the effects from intravenous SOD injections on convulsive and electroencephalographic manifestations of epilepsy in rats with amygdaline kindling and in those with generalized convulsive seizures induced by the drug corazole or by electric shock.

MATERIALS AND METHODS

The effect of SOD on amygdaline kindling was evaluated in 25 sexually mature male Wistar rats weighing 250-300 g. Intracerebral electrodes were implanted under Nembutal anesthesia (40 mg/kg intraperitoneally) by stereotaxic technique (the coordinates were determined using a stereotaxic atlas of the rat brain [12]). During the implantation operation, bundles each consisting of three nichro-

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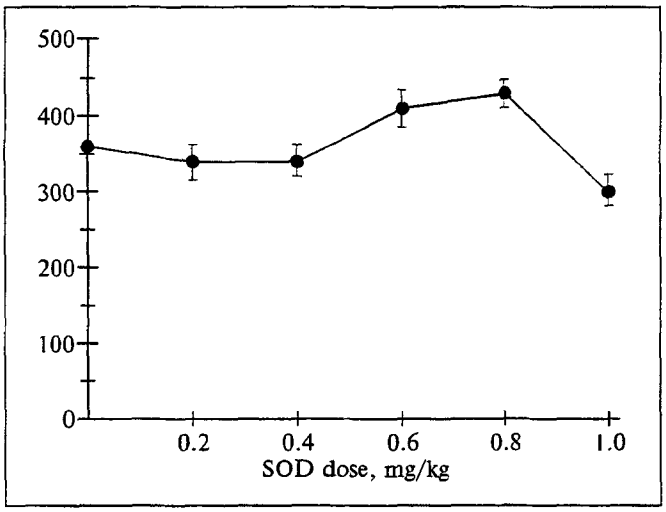

Fig. 1. Threshold values of initiating electrostimulation (ES) as a function of the SOD dose. *Ordinate*: mean thresholds for initiating ES, μA. The threshold in the control group corresponds to the zero SOD dose.

me electrodes insulated with fluoroplastic lacquer were inserted into the basolateral portions of both cerebral hemispheres. One of the electrodes was used for recording the electroencephalogram (EEG) and the other two for bipolar stimulation of the structure of interest. The reference electrode was a

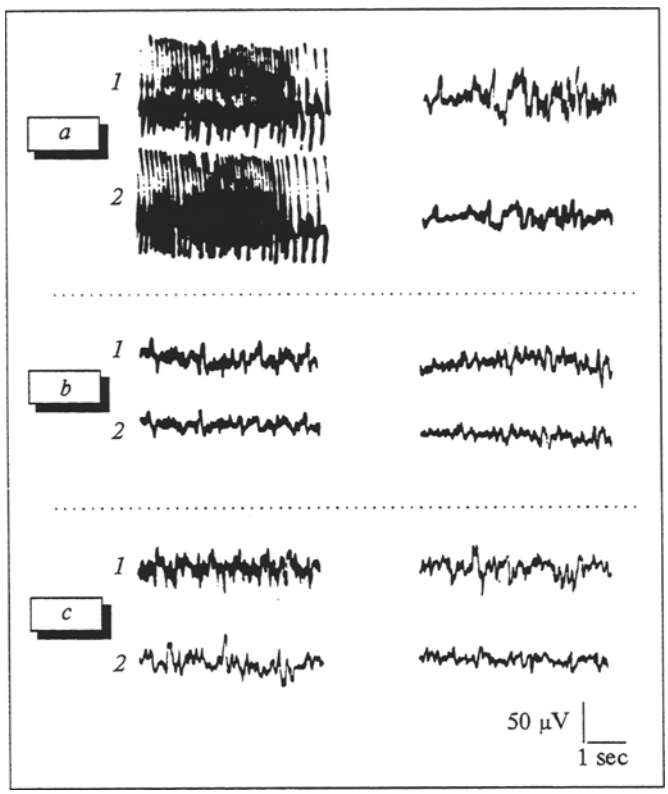

Fig. 2. Tracings of brain bioelectrical activity at the amygdala (*1*) and sensorimotor cortex (*2*). *Left*: tracings during a seizure; *right*: tracings during the interictal period. *a*) control; *b*) last (6th) day of SOD injections; *c*) 24 h after the discontinuation of SOD dosing.

gilded plate affixed to cranial bones under the aponeurosis. The outer electrode ends with plugs soldered to them were attached to the cranial surface with acrylic plastic.

One week after the operation, electrostimulation (ES) was started - one 10-second session per day using two-phase rectangular pulses delivered at a frequency of 100 Hz. The number of stimuli and the current strength were progressively increased until an afterdischarge appeared on the EEG. Stimulation was continued to the point of eliciting tonic-clonic seizures that caused the rat to fall. The rats which failed to attain this stage of epilepsy were excluded from the test group. The stages of epileptic seizures were classified according to Rasine [14]. When the 5th stage was reached, the rat was monitored during 2 weeks for stability of the elaborated seizures.

The SOD used for intravenous injections was obtained from bovine erythrocytes at the Research Institute of Extra Pure Preparations (St. Petersburg); its activity, as determined spectrophotometrically by the decrease in the reduction of nitro blue tetrazolium at 540 nm [2], was 300 U/mg protein. The rats with elaborated kindling were divided into 5 groups of 5 animals each. The first four groups were injected intravenously with a SOD solution in physiological saline in doses of 0.4, 0.6, 0.8, and 1 mg/kg body weight, respectively. The fifth, control group received physiological saline in the same volume (0.5 ml). ES causing stage 5 seizures was carried out 2 h before SOD injection, 5 min postinjection, and then every 10 min for a total of 2 h. All test groups received one SOD injection daily for 6 days.

To evaluate the effects of SOD on the seizures induced by electric shock and corazole, we used a total of 70 male Wistar rats, divided into 7 groups of 10 animals. The first six groups received SOD in a dose of 1.5 mg/kg at 180, 60, 30, 15, 5, and 1 min, respectively, before being subjected to electric shock or injected with corazole intraperitoneally (1 mg/kg). The seventh, control group received physiological saline in the same volume (0.5 ml).

The EEG was recorded with a Medicor electroencephalograph followed by recording on a magnetograph (Tesla). The EEGs obtained were processed using a signal analyzer (Bruel & Kjer).

RESULTS

The effects of different SOD doses on rats with amygdaline kindling were assessed by the degree and duration of seizures and by the threshold currents required to produce an afterdischarge. The

magnitude of seizures in response to ES was directly related to the SOD dose. Thus, the 0.6 mg/kg dose reduced the seizures, on average, to stage 3.8±0.4, the 0.8 mg/kg dose to stage 2.7±0.3 ($p<0.01$), and the 1 mg/kg dose to stage 1.3±0.2 ($p<0.001$). The control rats responded to each ES with stage 5 seizures.

Analysis of threshold currents for eliciting seizures in response to ES showed that the current had to be increased to initiate seizures after injecting SOD in the doses used (Fig. 1). The currents required to produce stage 5 seizures were 350±11, 418±21, and 440±25 μA after the SOD doses of 0.4, 0.6, and 0.8 mg/kg, respectively.

Substantial changes also occurred in the frequency of epileptic discharges recorded during interstimulus intervals after SOD dosing. The 0.4 mg/kg dose was not followed by a significant decrease in the frequency of epileptic spikes, whereas the 1 mg/kg dose led, 25 min postinjection, to a significant (and the greatest) fall in the frequency of these spikes to 26% of its baseline value; 45 min postinjection, epileptic spikes became more frequent without, however, reaching the baseline. Epileptic discharges were less frequent not only immediately after the discontinuation of SOD dosing (Fig. 2, *a* and *b*), but also during the following 3 days (Fig. 2, *c*).

A comparative analysis of the generalized epileptic seizures induced by corazole or electric shock in the control rats and in rats preinjected with SOD revealed that the patterns and intensities of these seizures and the durations of their phases were similar in the control and test groups, regardless of when corazole and electric shock were administered. This suggests that the epileptic states induced by different factors (kindling, corazole, electric shock) are organized by distinct mechanisms.

One possible explanation for the action of SOD observed in the kindling model of epilepsy is that this enzyme inhibits lipid peroxidation in the brain tissue. When a superoxide radical inducer was added to the incubation medium in various doses, none of the doses used was found to cause epileptic discharges [13]. The antiepileptic activity of SOD appears to be due to its ability to remove superoxide as an inactivator of nitric oxide, which, as has been shown recently [5], is an endogenous anticonvulsant. If so, then SOD may act within the vascular bed given the extremely low degree of its penetration through the blood-brain barrier [6,8]. The finding that SOD displays antiepileptic activity when administered by the intravenous route is encouraging for the development of new treatments for epilepsy.

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