Superoxide Production in Experimental Seizures in Cats

G. Conrad Bauknight Jr., MD; Enoch P. Wei, PhD; and Hermes A. Kontos, MD, PhD

Background and Purpose: Seizures cause cerebrovascular responses similar to those seen in conditions such as acute hypertension, ischemia/reperfusion, or fluid-percussion brain injury, which are associated with the generation of superoxide. Accordingly, we studied production of superoxide in experimental seizures.

Methods: Superoxide production was measured in anesthetized cats equipped with double cranial windows using the superoxide dismutase-inhibitable reduction of nitro blue tetrazolium as a measure of superoxide production. Seizures were induced by intravenous bicuculline. The contribution of hypertension associated with seizures was studied by maintaining arterial blood pressure constant by bleeding.

Results: Significant superoxide dismutase-inhibitable reduction of nitro blue tetrazolium indicative of superoxide production was found during seizures with or without control of arterial blood pressure (1.10±0.27 and 1.29±0.16 nmol/l/min, respectively).

Conclusions: The results show that experimental seizures are associated with superoxide generation that is independent of the rise in arterial blood pressure. It is likely that superoxide generation is due to the metabolic changes that occur during seizures. (Stroke 1992;23:1512-1514)

KEY WORDS • seizures • superoxide dismutase • cats

Experimental seizures are associated with marked cerebral arteriolar dilation,1 cerebral cortical hyperemia,2-3 altered vascular reactivity,1 and breakdown of the blood-brain barrier to macromolecules.2 These vascular abnormalities are similar to those seen in other experimental conditions that mimic human pathophysiological conditions. These include fluid-percussion brain injury,4 acute severe hypertension,5 and ischemia/reperfusion.6 In these conditions, it has been shown that superoxide is produced and that this oxygen radical and its derivatives are involved in the induction of the accompanying vascular abnormalities.7 Accordingly, we investigated the possibility that superoxide production also occurs in experimental seizures.

Materials and Methods

Experiments were carried out in adult cats of either sex anesthetized initially with 22 mg/kg i.m. ketamine followed by 50 mg/kg i.v. chloralose plus 750 mg/kg i.v. urethane. Additional doses of chloralose were administered later, based on motor responses to tail pinch, to maintain adequate surgical anesthesia. The animals were subjected to tracheostomy and were ventilated with a positive-pressure respirator connected to the tracheostomy tube. Volume and rate of respiration were adjusted to maintain Paco2 at about 30 mm Hg. Arterial blood gases and pH were measured with O2 and CO2 electrodes and a pH meter. Arterial blood pressure was monitored with a pressure transducer connected to a catheter placed into the aorta via the femoral artery. A large cannula was placed into the descending aorta via the other femoral artery and was used to induce controlled bleeding during seizures. Seizure activity was monitored by recording the electroencephalogram. Seizures were induced by the intravenous administration of 1.5–2 mg/kg bicuculline.

Superoxide production was measured as the superoxide dismutase (SOD)-inhibitable reduction of nitro blue tetrazolium (NBT), as described in detail previously.8 Briefly, the technique consisted of installing two cranial windows symmetrically to overlie each parietal cortex. The two windows did not communicate with each other. The space under each window was filled initially with artificial cerebrospinal fluid (CSF) with the same composition as endogenous CSF from cats. To measure superoxide production, the artificial CSF under each window was replaced with a solution of 2.4 mM NBT in artificial CSF. In one window we also placed 60 units/ml SOD (3,000 units/mg protein from bovine blood). NBT is a yellow, water-soluble dye that is reduced by superoxide and other substances to a blue insoluble form that precipitates. The reduction of NBT in the absence of SOD is due to superoxide and other reducing substances. In the window that contained SOD, superoxide was eliminated by this scavenging enzyme. Hence, by subtraction the SOD-inhibitable portion of NBT reduction was obtained and was used as a measure of superoxide production. The technique has been validated previously.8

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TABLE I. NBT Reduction in Cats With and Without Seizures

<table>
<thead>
<tr>
<th>Group</th>
<th>NBT reduction rate (nmol/min/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBT</td>
</tr>
<tr>
<td>Control</td>
<td>1.27±0.19</td>
</tr>
<tr>
<td>Seizures</td>
<td>2.73±0.53</td>
</tr>
<tr>
<td>Seizures with controlled blood pressure</td>
<td>2.39±0.53</td>
</tr>
</tbody>
</table>

NBT, nitro blue tetrazolium. Values are mean±SEM; n=6 for each group.
*p<0.05 different from corresponding value in control group.

At the end of the experiment, the excess NBT was washed away with fresh artificial CSF and the brain was perfused transcardially first with 0.9% NaCl solution and then with fixative consisting of a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer. Fixation by perfusion was used to eliminate reliably the red blood cells from the brain underlying the cranial window because hemoglobin may interfere with the spectrophotometric determination of NBT. This technique also allowed us to obtain a precisely measured superficial layer of the cortex for the measurement of NBT concentration. Following fixation by perfusion, the brain underlying each window was removed using a cork borer that had the same diameter as the window. The superficial 2 mm of the brain was then removed. We have shown previously that the deposition of NBT is fairly uniform in this thickness of brain.8 The precipitated reduced NBT was extracted with pyridine as described previously,8 and its concentration was determined spectrophotometrically at a wavelength of 530 nm using an extinction coefficient of 17.9 × 10³ M⁻¹ cm⁻¹.

The experimental design was as follows. A few minutes before the induction of seizures, after checking to ascertain that the cats were adequately anesthetized, they were subjected to skeletal muscle paralysis using 5 mg/kg i.v. gallamine triethiodide to inhibit the muscular movements from seizures. Three groups of animals were used. In the first group, superoxide production was measured under resting conditions without the induction of seizures. In the second group, seizures were induced with bicuculline and superoxide production was measured for 10 minutes during seizure activity. In the third group, the arterial blood pressure, which typically rises during seizures, was not allowed to increase above the baseline value by controlled bleeding via the arterial cannula placed into the aorta. Six cats were included in each group.

Statistical analysis was done by analysis of variance followed by t tests modified for multiple comparisons.

**Results**

Table 1 shows that seizures were associated with a marked increase in SOD-inhibitable reduction of NBT, indicative of superoxide production. The elimination of the associated hypertension during seizures did not alter significantly the production of superoxide.

In the first group, mean arterial blood pressure was initially 102±2.5 mm Hg and remained stable throughout the period of measurement of NBT reduction. In the second group, mean arterial blood pressure was initially 97±2 mm Hg and remained at that level during seizures.

**Discussion**

Our findings show that there is significant production of superoxide on the brain surface during experimental seizures. A second important finding was that superoxide production under these conditions did not depend on the increase in blood pressure. We found previously that acute elevations in arterial blood pressure in cats induce superoxide production in the brain.9,10 In those earlier experiments the increase in blood pressure was considerably higher than in the present experiments.9,10 Apparently, this accounted for the different effects of hypertension on superoxide production.

Because superoxide production during seizures was not due to the associated hypertension, it is likely that the increased metabolism and associated release of neurotransmitters might account for the increased superoxide production. During seizure activity, a number of systems that could lead to superoxide generation could be activated. Catecholamines released during seizures may undergo metal-catalyzed auto-oxidation, which produces superoxide as well as its derivatives hydrogen peroxide and hydroxyl radical.11-13 It is known that during seizures there is activation of phospholipases and a large increase in the concentration of free fatty acids including arachidonate.14,15 This fatty acid is metabolized via cyclooxygenase to produce eicosanoids. Cyclooxygenase also generates superoxide in the presence of suitable cofactors.16 Although the concentrations of glutamate and aspartate in brain can increase in certain types of seizures,17 the concentration of glutamate in the cortex actually decreases during bicuculline seizures18 while the concentration of aspartate does not change significantly.18 Therefore, the action of excitotoxic amino acids seems to be a less likely source of superoxide in seizures induced by bicuculline.

Because superoxide and its derivatives are very reactive and capable of causing tissue injury, the possibility might be profitably considered that they are the mediators of some of the vascular or neuronal abnormalities that occur during prolonged seizures.

**References**

8. Kontos HA, Wei EP, Jenkins LW, Powlishtock JT, Rowe GT, Hess ML: Appearance of superoxide anion radical in cerebral extracel-
In Dr. Kontos' continuing effort to elucidate the role of free radicals in the pathophysiological responses of the cerebrovasculature, Dr. Bauknight has provided evidence for superoxide generation during bicuculline-induced seizures in cats, independent of the concomitant arterial hypertension. This may not be surprising because the known cerebrovascular and metabolic responses of the brain during seizures provide many reasons to suspect increased superoxide generation during seizures. Brain oxygen consumption and cerebral blood flow increase dramatically, tissue hyperoxia occurs, neurotransmitter (e.g., catecholamine) turnover increases, and free fatty acids increase, all of which contribute to accelerated superoxide generation. What is interesting is Dr. Kontos' view of these phenomena. He hypothesizes that because cerebral hyperemia, altered cerebrovascular reactivity, and blood-brain barrier breakdown occur in seizures as they do in other cerebral insults (e.g., brain injury, acute, severe, arterial hypertension) in which there is evidence of superoxide generation, increased superoxide generation occurs in seizures. Intended or not, this view tacitly implies that superoxide generation is the sole basis for these cerebrovascular effects and negates a multifactorial approach to the problem (see Figure).

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