Brain Adenosine Triphosphate: Decreased Concentration Precedes Convulsions

Abstract. *The concentration of adenosine triphosphate in the brain decreased before the onset of generalized convulsions in unanesthetized rats subjected to acute hypoxia or treated with hydroxylamine or pentylenetetrazole (Metrazol). As the convulsive episode continued, adenosine triphosphate decreased further. Stimulation of adenosine triphosphate production forestalled its disappearance from the brain and delayed the development of seizure activity.*

Hypoxia and hyperbaric oxygenation decrease the concentration of adenosine triphosphate (ATP) in the brain and cause convulsions (*1*). We investigated the possibility that loss of brain ATP precedes the onset of convulsions. We performed a series of experiments on groups of fasted, male, Sprague-Dawley rats (150 to 200 g), subjected either to acute hypoxia (0.4 percent oxygen plus 99.6 percent nitrogen), to intraperitoneal injections of hydroxylamine (100 mg/kg), or to intramuscular injections of pentylenetetrazole (Metrazol, 120 mg/kg); control animals received equivalent doses of saline. All treated rats experienced convulsions. After each of the convulsive agents was administered, the intact, unanesthetized animals were decapitated at timed intervals before and after the onset of convulsions; the heads were immediately placed in liquid propane.

The average concentration of ATP in the brains, determined by methods described previously (*1*), was (mean ± S.D.) 2.03 ± 0.21 μmole per gram of brain (wet weight) ($N = 20$); this value is substantially less than that previously observed in ether-anesthetized, scalped rats (*1*) or in barbiturate-anesthetized, craniectomized rats (*1*). We attribute these differences both to the effects of the anesthesia and to the unavoidable, though slight, delay in tissue freezing inherent in the procedure used.

The concentration of ATP in the brain was uniformly and significantly decreased prior to the onset of the convulsions induced by each of the epileptogenic agents (Fig. 1). The average ATP concentrations in the brains immediately before and after convulsions produced by the three methods were 66.5 and 46.0 percent, respectively.

The occurrence of consistent reduction of cerebral energy reserves before the onset of induced seizures has not previously been reported. However, several investigators considered such a mechanism in the etiology of convulsive disorders (*2*). Reports of data obtained after initiation of the seizure process have appeared (*3*). As in our study, significant depletion of brain ATP was generally observed after seizures. For example, Klein *et al.* (*3*) measured the concentrations of nine different cerebral constituents in the brains of rats in which convulsions were induced by ten different epileptogenic agents. Of the cerebral constituents measured, only the ATP concentration varied consistently in one direction; it decreased after convulsions. The decreased concentration of ATP in the brain during seizures has been attributed to increased utilization of ATP, secondary to functional events associated with the seizure, which presumably exceeds the rate of ATP synthesis.

In further support of our hypothesis, two observations may be cited. (i) The amount of intracellular sodium of brain increases before the convulsion (*4*). (ii) The most common characteristic of the neuronal population which initiates the seizure discharge is an oscillating but progressive membrane depolarization leading to a sustained depolarization which is immediately followed by the seizure episode (*5*). Coirault and Jeanneton (*4*) have emphasized that seizures in general may be preceded by retention of intracellular sodium. This

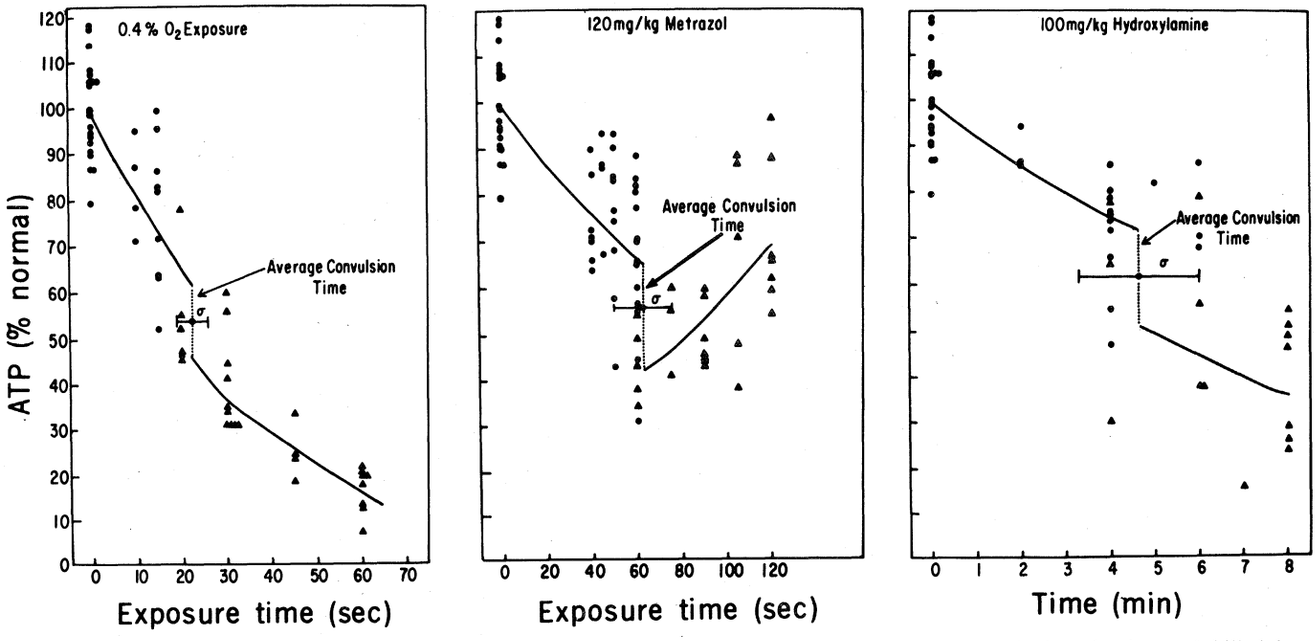

Fig. 1. The concentration of ATP in the brains of rats exposed to 0.4 percent oxygen, Metrazol, or hydroxylamine and killed before or after convulsion is plotted against the time to convulsion. The ATP concentrations were calculated as the percentage of normal [2.03 ± 0.21 μmole per gram of brain (wet weight)] for each convulsive agent. The solid curves were obtained from standard regression analysis of the data from rats in each group before and after convulsions. The average time to convulsion (± 1 standard deviation) is also shown. ●, Before convulsion; ▲, after convulsion.

suggests that fluctuations in the efficiency of sodium extrusion should be considered in relation to the variations in excitability which are frequently associated with the preconvulsive state. Adenosine triphosphate is essential for sodium extrusion from the cell (*6*). Thus, observed increases of intracellular sodium prior to seizures implicates decreased ATP as a possible mechanism whereby seizures are initiated. The neuronal membrane depolarization recorded before focal seizures is consistent with such a mechanism.

An apparent exception to our hypothesis was found in an article by Folbergrova *et al.* (*7*) in which it was reported that brain ATP remained unchanged before (and during) convulsions induced in mice by the intraperitoneal injection of methionine sulphoximine (MSO). In similar experiments with rats given MSO (500 mg/kg) intraperitoneally we found that convulsions, when present, occurred within 3 to 5 hours, shortly before the animals died. Brain ATP was measured 2.5 to 4.0 hours after the injection and before and after convulsion (Table 1, group A).

Methionine sulphoximine causes irreversible binding in vivo of ATP to brain glutamine synthetase (notably at noncholinergic nerve endings) in a molar ratio of up to 16:1 (*8*). Because similar irreversible binding of ATP might occur with other enzymes or even organelles, we attempted to ascertain whether such a mechanism might "sequester" intact, acid-extractable ATP under the influence of MSO. The expected cellular response to a sequestration of intact ATP into a nonfunctional compartment could account for the increased amount of total ATP in the brains in animals before convulsions. This explanation was tested by assuming that if a significant complement of ATP was indeed rendered unavailable for energy-requiring processes by MSO, it should similarly be protected from rapid autolytic degradation. Such proved to be the case (Table 1, group B).

These experiments support our general observation that the onset of induced seizures is preceded by a profound deficit of functional energy reserves. If, after 20 seconds of autolysis, the difference in brain ATP between control animals (43.3 percent) and those treated with MSO and killed before convulsion (89.4 percent) is an approximate reflection of sequestered ATP, then 46.1 percent of the ATP in the treated animals was in a sequestered "nonfunctional" state. When this value is subtracted from the observed total ATP concentration in rats treated with MSO and killed at intervals before and after convulsion (to give an estimate of readily utilizable ATP), the resultant values—67.1 and 42.7 percent of normal, respectively—are in agreement with the average concentration of ATP before (66.5 percent) and after (46 percent) convulsions in the hydroxylamine, Metrazol, and acute hypoxia experiments reported above.

If decreased brain ATP precedes the onset of overt seizures, then stimulation of ATP production should decrease the rate of ATP disappearance, and thereby delay the onset of convulsive activity.

Table 1. Effect of methionine sulphoximine (MSO) on the concentration of ATP in the brain immediately before and after convulsion. (Group A) The animal was decapitated directly into liquid propane. (Group B) The head was placed in liquid propane 20 seconds after the animal was decapitated (autolysis). The average concentration of ATP in the control brains was 2.03 ± 0.21 μmole per gram of brain (wet weight). The results are expressed as the mean ± standard deviation.

Control (% of average)	Before convulsion (% of control)	After convulsion (% of control)
	Group A	
100 ± 10.2	113.2 ± 11.3*	88.8 ± 13.2*
	Group B	
43.3 ± 15	89.4 ± 12.5*	71.3 ± 17.3†

* $P < .01$. † $P < .05$.

In experiments on acute hypoxia, we observed that the rate of brain ATP disappearance was inversely related to the oxygen concentration in the chamber. Therefore, our hypoxia studies were repeated except that we used 0.8 percent O_2 plus 99.2 percent N_2 in one experiment and 2 percent O_2 plus 98 percent N_2 in another. The animal chamber was first flushed for 10 to 15 minutes with the N_2 mixture, and then O_2 concentrations within were measured with a Clarke oxygen electrode. Brain ATP was determined at intervals consistent with the increased time to convulsions (Fig. 2). As the oxygen tension increased, the ATP increased and the time to convulsion also increased.

Exogenous succinate stimulates brain ATP production in animals (*9*). A group of fasted rats was given intraperitoneal injections of sodium succinate (12 mmole/kg, 0.4*M*, *p*H 6.4) 50 minutes before the intramuscular injection of the convulsive agent Metrazol (120 mg/kg). The time to convulsions was recorded in one group of animals. The concentration of ATP in the brain was determined in a second group of animals 65 seconds after the Metrazol injection. Identical experiments were performed in two groups of animals receiving only the Metrazol injection. In the 35 animals receiving Metrazol only, convulsions occurred at 62.7 ± 11.9 seconds, whereas 21 rats given prior treatment with succinate

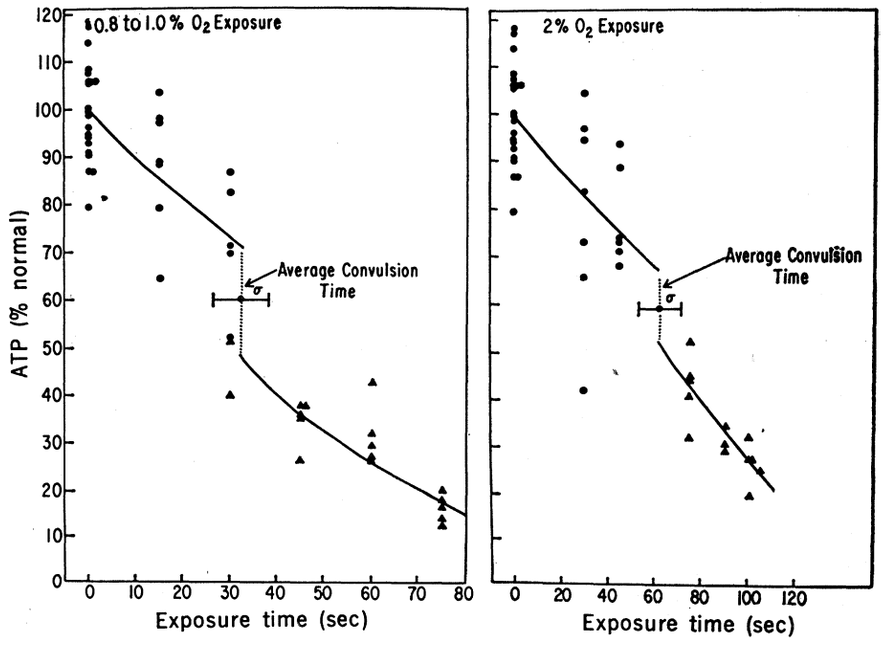

Fig. 2. The concentration of ATP in the brains of rats exposed to 0.8 to 1 percent or 2 percent oxygen and killed before or after convulsion is plotted against the time to convulsion. The average ATP concentration was 2.03 ± 0.21 μmole per gram of brain (wet weight). ●, Before convulsion; ▲, after convulsion.

convulsed at 90.9 ± 18.7 seconds ($P < .0005$). Furthermore, 65 seconds after the administration of Metrazol, brain ATP in 25 untreated rats, killed before convulsion, was 70.9 ± 16.6 percent of normal values, in contrast to a value of 90.7 ± 12.3 percent observed in 24 rats given prior treatment with succinate ($P < .0005$). Thus, the relation between onset of seizure and ATP concentration in the brain is unaltered under these conditions. These data support the proposal that decreased brain ATP concentrations precede the onset of convulsions.

The fact that the concentration of ATP in the brain decreases before the onset of seizures in these animals does not imply that decreased brain ATP is the cause of seizures. It does imply that decreased ATP in the brain is a common denominator in various seizure states. Numerous metabolic derangements induced by convulsant drugs might be expressed as a depletion of cerebral ATP. Any agent that markedly interferes with the substrate supply, major enzyme systems, cofactors, and the electron transport chain (the major source of ATP production under aerobic conditions), or that grossly stimulates hydrolysis of ATP by way of increased adenosine triphosphatase activity, could lead to decreased ATP concentrations in the brain. Various convulsive agents decrease nicotinamide-adenine dinucleotide (*10*) (essential for 9/11 of ATP production in aerobic metabolism); inhibit glutamic decarboxylase (*11*) and γ-aminobutyric acid transaminase (*12*), both of which have been implicated as critical enzyme systems in providing essential substrate for ATP production under conditions of stress (*9*); and stimulate membrane adenosine triphosphatase activated by sodium and potassium ions (*13*). These observations support the hypothesis that decreased ATP concentration precedes and perhaps contributes to the onset of convulsions.

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References and Notes

1. A. P. Sanders, D. M. Hale, A. T. Miller, Jr., *Amer. J. Physiol.* **209**, 438 (1965); *ibid.*, p. 443; A. P. Sanders, I. H. Hall, B. Woodhall, *Proc. Soc. Exp. Biol. Med.* **121**, 32 (1966); *Proceedings of the Third International Conference on Hyperbaric Medicine*, I. W. Brown and B. G. Cox, Eds. (National Academy of Sciences–National Research Council, Publ. 1404, Washington, D.C., 1966), p. 73; A. P. Sanders and I. H. Hall, *Proc. Soc. Exp. Biol. Med.* **125**, 716 (1967).
2. L. S. Wolfe and K. A. C. Elliott, in *Neurochemistry*, K. A. C. Elliott, H. Page, J. H. Quaster, Eds. (Thomas, Springfield, Ill., 1962), p. 697.
3. J. R. Klein and N. S. Olsen, *J. Biol. Chem.* **167**, 747 (1947); N. Allen, *Clin. Neurosurgery* **14**, 386 (1967); F. N. Mianrd and R. V. Davis, *J. Biol. Chem.* **237**, 1283 (1962); W. E. Stone, J. E. Webster, E. S. Gurdjian, *J. Neurophysiol.* **8**, 233 (1945); B. G. Leonard, *Biochem. Pharmacol.* **14**, 1293 (1965); W. E. Stone, J. R. Tews, E. N. Mitchell, *Neurology* **10**, 241 (1960); R. M. C. Dawson and D. Richter, *Amer. J. Physiol.* **160**, 203 (1950); B. Sacktor, J. E. Wilson, C. G. Teikert, *J. Biol. Chem.* **241**, 5071 (1966); L. J. King, O. H. Lowry, J. V. Passoneau, V. Venson, *J. Neurochem.* **14**, 599 (1967).
4. H. F. Colfer and H. E. Essex, *Amer. J. Physiol.* **150**, 27 (1947); R. Coirault and C. Jeanneton, *Epilepsie et Metabolism Cellulaire* (Maloine, Paris, 1959); D. M. Woodbury, L. T. Rollius, M. D. Gardner, W. L. Hirschi, J. R. Hogan, M. L. Rallison, G. S. Tanner, S. A. Brodie, *Amer. J. Physiol.* **192**, 79 (1958).
5. H. Matsumoto and C. A. Marsan, *Exp. Neurol.* **9**, 286 (1964); *ibid.*, p. 305; M. Sawe, S. Kaji, K. Usuki, *Clin. Neurophysiol.* **19**, 248 (1965); D. E. Prince and B. J. Wilder, *Arch. Neurol.* **16**, 194 (1967).
6. A. L. Hodgkin, *Proc. Roy. Soc. London Ser. B* **148**, 1 (1958).
7. J. Folbergrova, J. V. Passoneau, O. H. Lowry, D. W. Schulz, *J. Neurochem.* **16**, 191 (1969).
8. R. A. Ronzio, W. B. Rowe, A. Meister, *Biochemistry* **8**, 1066 (1969); E. DeRobertis, O. Z. Sellinger, R. L. Arnaiz, M. Alberici, L. M. Zeiher, *J. Neurochem.* **14**, 81 (1967).
9. A. P. Sanders, W. D. Currie, B. Woodhall, *Proc. Soc. Exp. Biol. Med.* **130**, 1021 (1967).
10. P. S. Schein, *ibid.* **131**, 517 (1969).
11. M. Alberici, G. R. L. Arnaiz, E. DeRobertis, *Biochem. Pharmacol.* **18**, 137 (1969).
12. C. F. Baxter and E. Roberts, *Proc. Soc. Exp. Biol. Med.* **101**, 811 (1959).
13. B. K. Pal and J. J. Ghosh, *J. Neurochem.* **15**, 1243 (1968).
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Electrophysiological Evidence for Binocular Disparity Detectors in Human Visual System

Abstract. *Evoked potentials have been recorded from humans in response to two moving gratings presented stereoscopically to both eyes. The amplitude of the evoked potential is greater when the two gratings have slightly different spatial frequencies, which produces an apparent inclination of the binocularly fused image. The amplitude of the response is correlated with the degree of the perceived inclination.*

Binocular vision is a mechanism for depth perception. The stimulus for the stereoscopic experience of depth is the geometrical disparity between the images, in the two eyes, of objects located at different distances from the observer. Neurons have recently been found in the cat visual cortex which are mostly activated when the two eyes are stimulated by equal stimuli in two noncorresponding areas of their retinas (*1*). These neurons have been interpreted to be responsible for binocular depth perception. We have attempted to correlate the psychophysical findings on binocular depth perception in man with the electrical activity of the brain by recording the visual evoked potentials.

Recently, evoked potentials have been recorded from the human scalp when a moving grating is used as a stimulus (*2*). The response obtained was a rather simple waveform and it originates mainly in the central nervous system. This stimulus is particularly suitable for our purposes, because the parameters of the response can be readily evaluated and because gratings of different spatial frequencies presented stereoscopically to the two eyes supply a convenient pattern to generate binocular depth perception.

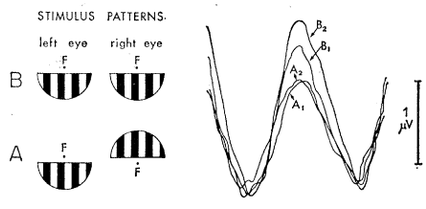

Fig. 1. Average evoked responses from the occipital area of scalp. One electrode was positioned just above the inion; the other was displaced 6 cm to the left. The responses A_1 and A_2 were recorded with two square-wave gratings that were not binocularly superimposed (A). For A_1 the two gratings had the same spatial frequency (1.33 cycle/deg); for A_2 the two spatial frequencies were 1.33 (right eye) and 1.60 cycle/deg (left eye). The responses B_1 and B_2 were recorded with two gratings that appeared binocularly superimposed (B) and had spatial frequencies as for A_1 and A_2, respectively. The gratings were semicircular with a diameter of 5.5 deg. In the schemes *F* indicates the fixation point. The contrast of the gratings was about 1.5 log units above threshold.