SHORT COMMUNICATIONS

The effect of experimentally induced seizures on the concentration of tissue and blood magnesium

(Received 13 November 1971; accepted 14 December 1971)

Previously we have studied some of the changes in labile components of the brain which occur following seizures induced by electroshock, leptazol and high intensity sound.^{1,2}

Magnesium is an essential cofactor for many enzymes associated with neuronal activity.³ Furthermore, hyperexcitability and convulsions occur in rodents maintained on a magnesium deficient diet.^{4–7} This investigation is an attempt to ascertain the role of magnesium in convulsions induced by the three types of stimuli used in the previous studies.

Methods

Albino rats (female, 100-120 g) of the Wistar strain were used. Convulsions were induced in groups of at least six animals by leptazol (100 mg/kg i.p.), high intensity sound or electroshock using the methods described previously. At the appropriate phase of the seizure (shown in Results) the rats were decapitated and blood collected from the cut carotid and jugular vessels. Brain, heart, kidney, spleen, skeletal muscle and spinal cord were removed, cleared of connective tissue and dried in a hot air oven to constant weight. One-ml aliquots of the sera were similarly dried to constant weight.

The dried tissues were extracted with 2 N nitric acid (10 ml) by grinding in a mortar with acid washed sand. The resulting homogenates were allowed to stand at room temperature for 24 hr, heated at 90° for 2 hr and centrifuged (500 g for $10 \min$). Aliquots of the supernatant fluid were removed for the determination of magnesium by the method of Bohuon. To assess whether any change in the blood volume occurred during the seizures, 1-ml samples of blood were centrifuged in haematocrit tubes at 2000 g for $30 \min$.

Results and discussion

All three stimuli produced a significant increase in the serum magnesium concentration (Table 1). The magnesium concentration of the spleen decreased in those animals subjected to maximal convulsions with leptazol (Table 2). No change occurred in the magnesium concentration of the other tissues nor could any change be detected in the cerebral hemispheres, mid brain or cerebellum.

These experiments suggest that, despite hyperexcitability and sedation being normally associated with decreased⁴⁻⁷ or increased⁵ tissue magnesium levels respectively, there is no correlation between tissue magnesium and experimentally induced seizures. The plasma concentration of magnesium increased during the full tonic phase of the seizure irrespective of the nature of the seizure stimulus. Magyarlaki¹⁰ also found that the concentration of this ion was increased in the plasma of rats following seizures induced by strychnine and leptazol.

The increased serum magnesium concentration may be partly attributed to the slight haemoconcentration which occurs during maximal seizure activity (Table 3). The slight decrease in the magnesium

TABLE 1. THE SERUM MAGNESIUM CONCENTRATION DURING EXPERIMENTALLY INDUCED SEIZURES

	Leptazol	High intensity sound	Electroshock
Control group Experimental group	0.675 ± 0.02 0.900* ± 0.02	$ \begin{array}{r} 0.758 \pm 0.03 \\ 1.295 + 0.01 \end{array} $	$\begin{array}{ccc} 0.750 & \pm & 0.03 \\ 0.967 \dagger & \pm & 0.02 \end{array}$

Rats (six per group) killed during the full tonic phase of the seizure. Each result represents the mean \pm S.E.M. expressed as m-equiv./l. serum. The significance of the difference between the control and experimental group shown by *P < 0.01, \uparrow P < 0.001.

Table 2. The magnesium concentration of the spleen during experimentally induced seizures

	Leptazol	High intensity sound	Electroshock
Control group Experimental group	13·34 ± 0·6 7·65* ± 0·7	$ \begin{array}{c} 10.58 \pm 0.4 \\ 9.56 \pm 0.7 \end{array} $	14·42 ± 0·1 15·97 ± 0·2

content of the spleen may also play some part; we found that the spleen volume decreased during leptazol induced seizures, but not during electroshock (unpublished) and this may contribute to the increased magnesium concentration in the serum in some cases. Another investigator showed that electrical stimulation of skeletal muscle causes a marked elevation of serum magnesium¹¹ although no change in the concentration of this ion was found in the skeletal muscle during the present study.

TABLE 3. THE DEGREE OF HAEMOCONCENTRATION DURING EXPERIMENTALLY INDUCED SEIZURES

	Leptazol	High intensity sound	Electroshock
Control group Experimental group	$\begin{array}{c} 42.0 \pm 0.8 \\ 45.0 \pm 2.2 \end{array}$	$\begin{array}{c} 42.0 \pm 0.4 \\ 46.8 \pm 1.1 \end{array}$	41·9 ± 1·2 46·1 ± 2·0

Results expressed as mean percentage red blood cells \pm S.E.M. Details otherwise as shown in Table 1.

It can be concluded that the raised serum magnesium concentrations are a consequence of peripheral changes which occur irrespective of how the seizure is initiated.

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REFERENCES

- 1. M. G. Palfreyman and B. E. Leonard, Biochem. Pharmac. 21, 355 (1971).
- 2. B. E. LEONARD and M. G. PALFREYMAN, Biochem. Pharmac. 21, 1205 (1972).
- 3. W. E. C. WACKER and B. L. VALLEE, New Engl. J. Med. 259, 431, 475 (1958).
- 4. H. D. KRUSE, E. R. ORENT and E. V. McCollum, J. biol. Chem. 96, 519 (1932).
- 5. E. WATCHORN and R. A. McCance, Biochem. J. 31, 1379 (1937).
- 6. E. V. Tufts and D. M. Greenberg, J. biol. Chem. 122, 673 (1938).
- 7. I. MACINTYRE and D. DAVIDSON, Biochem. J. 70, 456 (1958).
- 8. C. Bohuon, Clin. Chim. Acta 7, 811 (1962).
- 9. O. H. PECK and J. J. MELTZEK, J. Am. med. Ass. 67, 1131 (1916).
- 10. A. MAGYARLAKI, Kiseri. Orvostud. 14, 375 (1962).
- 11. L. WACKER, Klin. Wschr. 8, 244 (1929).
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Biochemical Pharmacology, Vol. 21, pp.1370-1373. Pergamon Press, 1972. Printed in Great Britain.

Increased vascular permeability and activation of plasminogen by the trypsin-like esterases from the mouse submandibular gland

(Received 29 July 1971; accepted 30 November 1971)

The submandibular gland of mouse contains a variety of enzymatically and biologically active substances, which include esteroproteinolytic enzymes, ¹⁻⁹ kallikrein ^{12,13} and a nerve growth promoing protein (NGF). ^{14,15} All of these are also capable of splitting N-benzoyl-arginine ethyl ester (BAEE) which is a widely used synthetic substrate for the determination of trypsin-like enzymatic activity. The relationships between these substances are not, however, clear-cut and well-established.

Abbreviations used: AMCA = p-Aminomethyl-cyclohexane carboxylic acid.