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SOME NEUROCHEMICAL CORRELATES OF CONVULSIVE ACTIVITY IN THE RAT BRAIN

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Abstract—The effects of seizures induced by leptazol, electroshock and high intensity sound were studied on some parameters of glycolysis in the rat brain. Whereas the glycolytic flux was increased to approximately the same extent irrespective of the means by which the seizure was induced, leptazol produced a biphasic change in total brain hexokinase activity. The activity of this enzyme was increased during the initial stages of the seizure but decreased with increasing seizure severity. The possible reasons for the effects are discussed.

THERE is evidence that hexokinase and phosphofructokinase are implicated in the regulation of the rate of brain glycolysis under normal conditions[1–3] and during the period of hyperactivity associated with chemically induced convulsions.[4] We have now compared the effects on glycolysis in the rat brain associated with seizures induced by various means, in the hope of distinguishing the neurochemical changes which initiate seizures from those which result from them.

METHODS

Female albino rats (100–120 g) of the Wistar strain were used. Convulsions were induced in groups of at least six animals by electroshock, leptazol or by high intensity sound. Electroshock convulsions were induced by applying a current of 25 mA for 0·2 sec duration through ear electrodes using an apparatus similar to that described by Woodbury and Davenport.[5] Chemically induced convulsions were elicited by giving the $C.D_{100}$ of leptazol (100 mg/kg i.p.). Rats were subjected to high intensity sound (100 db) for 90 sec by means of an electric door bell placed approximately 30 cm above the animals which were confined in a glass tank of 30 cm^3. This resulted in a full extensor tonic reflex in about 75 per cent of the rats of this strain. Those which responded in this way were kept for at least 1 week before being used for an experiment. In one series of experiment, a comparison was made between Wistar rats which were highly susceptible to audiogenic seizures (strain A) and rats of the same strain but from a different source which were not susceptible to audiogenic seizures (strain B). Rats of strain B formed a control group for this series of experiments.

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For the determination of labile constituents during phases of the seizures induced by these three means, the rats were killed by totally immersing them into liquid nitrogen. The brains were rapidly removed while still frozen, crushed on a metal anvil of the type described by Stone,[6] and triturated in a cooled glass mortar with a protein precipitant, generally 5 per cent (w/v) trichloroacetic acid (TCA). After centrifugation (600 *g* for 10 min) aliquots of the supernatant fraction were removed for the enzymatic determination of lactate,[11] fructose-1,6-diphosphate[10] and glucose-6-phosphate.[9]

Blood and brain glucose determinations. The animals were carefully held in liquid nitrogen so that only the head was immersed. After thorough freezing, the animals were decapitated and samples of blood were collected from the severed carotid and jugular vessels. After precipitation with perchloric acid, blood glucose was determined by the enzymatic method of Hugget and Nixon.[7] The frozen brains were rapidly removed, crushed and triturated with a mixture of barium hydroxide and zinc sulphate as described by Gey.[8] After centrifugation at 20,000 *g* for 15 min in M.S.E. "High Speed" 18 centrifuge, aliquots of the supernatant fraction were taken for the enzymatic determination of glucose.[7]

Brain hexokinase activity was determined on samples (50–100 mg) of unfrozen brain tissue. The tissue was homogenized in 10 ml of cold 0·1 M phosphate buffer (pH 8·0) using a Potter–Elvehjem homogenizer and the total hexokinase activity determined by the method of Bennet and co-workers.[12]

In some experiments the rat brains were dissected into three main anatomical areas; the cerebral hemispheres, cerebellum, and the diencephalon. The diencephalon was taken to be the area below the lateral ventricles and between the striatum and the corpora quadrigemina.

In all experiments, groups of rats were killed at the pre-convulsive, clonic, full tonic and sometimes in the catatonic phase which followed the seizure. Details of these stages, and the approximate duration of their onset, are given in the Results.

The results were analysed statistically using Students' *t*-test.

RESULTS

Nature of the convulsions

The different phases of the seizures induced by the three stimuli may be summarized thus:

Pre-convulsive phase. This refers to the time between application of the stimulus and the onset of any sign of behavioural hyperexcitability. The animals were slightly depressed at this time. This phase was clearly apparent after leptazol and high intensity sound but was of such short duration after electroshock that it was difficult to identify.

Clonic phase. This stage marked the first sign of behavioural hyperexcitability. Some 30–40 sec after the injection of leptazol the rat showed small myoclonic jerks and its back became arched. This was immediately followed by clonic movement of the limbs. Following high intensity sound stimulation, the rat showed a fear response—it walked backwards away from the stimulus and showed increased stereotyped face washing and increased locomotor activity. This was succeeded by the "wild running" phase which started 35–55 sec after the commencement of the stimulus. During this

TABLE 1. CHANGES IN GLUCOSE, GLUCOSE-6-PHOSPHATE, FRUCTOSE DI PHOSPHATE AND LACTATE DURING SEIZURE ACTIVITY

Metabolite	Control	Phase of Seizure		Tonic	Catatoric
		Preconvulsive	Clonic		
		High Intensity Sound			
Blood Glucose	0·894 ± 0·003	0·896 ± 0·002	—	0·810 ± 0·004	1·201‡ ± 0·008
Brain Glucose-6-phosphate	0·284 ± 0·019	0·287 ± 0·017	—	0·081‡ ± 0·015	0·538† ± 0·057
Fructose-diphosphate	0·254 ± 0·019	0·201* ± 0·016	—	0·122‡ ± 0·011	—
Lactate	0·065 ± 0·004	0·094† ± 0·004	—	0·081* ± 0·009	0·094† ± 0·008
	1·631 ± 0·16	3·683‡ ± 0·19	—	3·830‡ ± 0·09	3·250‡ ± 0·34
		Electroshock			
Blood Glucose	0·895 ± 0·005	0·892 ± 0·001	—	0·892 ± 0·008	0·950 ± 0·009
Brain Glucose-6-phosphate	0·284 ± 0·019	0·318 ± 0·011	—	0·111‡ ± 0·017	0·355† ± 0·016
Fructose-diphosphate	0·253 ± 0·020	0·193* ± 0·019	—	0·150‡ ± 0·019	—
Lactate	0·068 ± 0·004	0·116‡ ± 0·018	—	0·088* ± 0·015	0·087* ± 0·018
	1·670 ± 0·20	3·075‡ ± 0·14	—	3·088‡ ± 0·44	3·861‡ ± 0·24
		Leptazol			
Blood Glucose	0·899 ± 0·006	0·900 ± 0·005	0·950 ± 0·004	0·930 ± 0·010	—
Brain Glucose-6-phosphate	0·285 ± 0·021	0·303 ± 0·014	0·284 ± 0·013	0·049‡ ± 0·005	—
Fructose-diphosphate	0·254 ± 0·019	0·284 ± 0·012	0·194 ± 0·013	0·064‡ ± 0·007	—
Lactate	0·065 ± 0·004	0·120‡ ± 0·01	0·110‡ ± 0·009	0·074 ± 0·005	—
	1·700 ± 0·22	2·578‡ ± 0·51	2·613‡ ± 0·33	2·536‡ ± 0·24	—

Each result represents the mean ± S.E.M. of at least six animals. All results expressed as μmole/g wet weight brain or m-mole/ml blood. The significance of the difference between the control and the experimental groups shown by

* $P < 0.05$.

† $P < 0.02$.

‡ $P < 0.01$.

period the rat ran wildly round the cage trying to escape from the stimulus. The "wild running" phase was succeeded by clonic movements of the limbs. The clonic phase was hardly noticeable following an electroshock stimulus of the intensity used in these experiments.

Tonic phase. This stage occurred irrespective of the means used to initiate the seizure. It was characterized by a fore and hind leg extension, increased muscle tone, loss of righting reflex and often a "Straub" tail. Frequently, this phase was succeeded by opisthotonus. The tonic phase occurred 50–90 sec after leptazol, 40–60 sec after high intensity sound but only 3–5 sec after electroshock.

Catatonia occurred after the full tonic phase of electroshock or sound induced convulsions. The animals were immobile with skeletal muscles in a state of tension. This period occurred 30–60 min after the tonic phase. Most of the animals given leptazol died after the full tonic phase.

The sequential changes in the concentrations of glucose, glucose-6-phosphate and fructose-1,6-diphosphate during seizure activity are shown in Table 1.

At the onset of seizure activity induced by any of the stimuli no change was found in the brain glucose concentration. However, as the severity of the seizure progressed to the extensor tonic stage, the brain glucose concentration decreased by 83, 72 and 62 per cent after leptazol, high intensity sound and electroshock respectively. During the post-ictal catatonic phase induced by high intensity sound and by electroshock, the brain glucose concentration increased slightly above the control level. Changes in the blood glucose concentration were only significant during the catatonic phase which followed audiogenic seizures when the blood glucose concentration was increased by approximately 34 per cent. The concentration of glucose-6-phosphate decreased significantly during the extensor tonic phase only, irrespective of how the seizure had been initiated. In contrast, fructose-1,6-diphosphate levels increased during the preconvulsive phase of seizure activity and remained elevated throughout the remainder of the convulsion.

A significant increase in the concentration of brain lactate occurred during the preconvulsive phase of the seizure irrespectively of how it was initiated (Table 1). The concentration of this metabolite remained elevated during the period of catatonia which followed the convulsions initiated by electroshock and high intensity sound.

Leptazol was found to have a small but significant effect on the activity of brain hexokinase (Table 2). In the initial phase of the seizure, hexokinase activity was found to increase whereas during the full extensor tonic phase of the seizure, its activity was slightly inhibited. No statistically significant changes in hexokinase activity

TABLE 2. CHANGES IN TOTAL BRAIN HEXOKINASE ACTIVITY INDUCED BY LEPTAZOL

Brain area	Control	Phase of seizure		
		Preconvulsive	Clonic	Tonic
Cerebral hemispheres	1240 ± 50	*1430 ± 57	*1398 ± 52	*1026 ± 43
Midbrain	1290 ± 56	*1450 ± 60	*1433 ± 84	* 995 ± 50
Cerebellum	1364 ± 60	1490 ± 74	1387 ± 121	*1022 ± 61

Results expressed as mean ± S.E.M. for at least six animals. The significance of the difference in activity between the control and the experimental groups shown as $*P < 0{\cdot}05$.

could be detected in rats which had been subjected to electroshock or high intensity sound seizures (Table 3).

TABLE 3. ABSENCE OF CHANGE IN TOTAL BRAIN HEXOKINASE ACTIVITY AFTER HIGH INTENSITY SOUND

Brain area	Control	Phase of seizure			
		Preconvulsive	Clonic	Tonic	Catatonic
Cerebral Hemispheres	1443 ± 58	1381 ± 70	—	1418 ± 48	1355 ± 69
Midbrain	1283 ± 53	1245 ± 35	—	1200 ± 41	1313 ± 33
Cerebellum	1261 ± 45	1304 ± 64	—	1295 ± 42	1335 ± 81

In the experiment in which rats were subjected to electroshock the values for hexokinase activity of the control were 1199 ± 62, 1047 ± 102 and 1074 ± 90 for the hemispheres, midbrain and cerebellum respectively. Following electroshock, no significant differences were found between the experimental and the control groups.

The results represent the mean ± S.E.M. of at least six animals. The activity is expressed as μmole glucose metabolized/g wet wt. brain/hr.

Some experiments were carried out in an attempt to determine how the activity of brain hexokinase could be affected by leptazol. One possibility was that leptazol seizures, because of their severity, could produce anoxia which might not have been so marked after audiogenic or electroshock convulsions. However, when rats were subjected to anoxia in a container through which nitrogen was flushed until the animals were unconscious and cyanosed, no detectable change in total brain hexokinase activity could be found. The hexokinase activity (as μmoles glucose utilized/g brain/hr) of the control rats was 946 ± 40 and for those rats undergoing anoxia 950 ± 60. Furthermore, leptazol in concentrations of up to 10 mg/ml, did not inhibit the activity of brain hexokinase *in vitro* nor did it affect that of a purified yeast preparation of this enzyme. These results suggest that leptazol may interfere with some co-factor which is important for hexokinase activity. Such a view is supported by the finding that hexokinase activity in animals which had been treated with leptazol was not inhibited if 2-mercaptoethanol had been added to the incubation mixture (Table 4).

TABLE 4. THE EFFECT OF 2-MERCAPTOETHANOL ON THE HEXOKINASE ACTIVITY OF RATS TREATED WITH LEPTAZOL

Tissue	Control rats		Leptazol treated rats	
	Untreated	Plus mercaptoethanol	Untreated	Plus mercaptoethanol
Cerebral Hemispheres	1223 ± 60	2050 ± 97	1001 ± 55	2041 ± 100
Midbrain	1054 ± 45	2039 ± 106	*860 ± 49	2030 ± 98
Cerebellum	1011 ± 50	1901 ± 87	*875 ± 53	1921 ± 168

0·1% (w/v) of 2-mercaptoethanol was added to the buffer medium. An equivalent volume of phosphate buffer was added to the "untreated" groups. Difference between the untreated control and leptazol treated groups significant at *$P < 0.05$. Treated rats killed during the tonic phase of the seizure.

These findings suggest that the changes in the activity of hexokinase are a consequence of the action of leptazol itself rather than of the convulsive state it produces. Such a view is further substantiated by the finding that rats which had been pretreated with an anaesthetic dose of sodium amylobarbitone (80 mg/kg i.p.) so that the convulsions due to leptazol were prevented, showed the same degree of hexokinase inhibition (approximately 20 per cent) to that found in rats given leptazol alone. However, when iodoacetate was added to the incubation medium containing homogenates from either untreated or leptazol treated rats, it did not prevent the inhibition of hexokinase caused by leptazol (Table 5).

TABLE 5. THE EFFECT OF IODOACETATE ON HEXOKINASE ACTIVITY

Concentration of iodoacetate (mg/ml buffer)	Control homogenate	Homogenate + iodoacetate	Homogenate from leptazol treated rat + iodoacetate
0·01	1103	1090	980
0·10	1201	1182	1003
1·0	1213	1190	991
10·0	999	930	902

Each result is the mean of three experiments. Values expressed as μmoles glucose utilized/g brain/hr. After giving leptazol, the rats were killed during the tonic phase of the seizure.

DISCUSSION

It is well established that the brain is dependent for its normal functions on an adequate supply of glucose.[13] During experimentally induced convulsions most investigators have reported a decrease in the concentration of this metabolite,[14–19,27] although some have reported no change[20–22] or even an increase in glucose during certain types of experimentally induced convulsions.[19,23]

In the present investigation, brain glucose concentrations were markedly decreased only during the tonic phase of the seizure, irrespective of how the seizure was initiated. Since hypoglycaemia did not occur concomitantly with the fall in brain glucose this decrease is probably a consequence of the glucose being utilized at a greater rate than it can be transported into the brain from the blood. During the catatonic phase which occurs following the sound induced seizures, both brain and blood glucose levels were raised. Other investigators have reported similar effects.[24]

The increase in the concentration of fructose-1,6-diphosphate which occurs soon after the onset of seizure activity supports the hypothesis that phosphofructokinase is an important enzyme in controlling the rate of brain glycolysis;[2,3] it would seem likely that the activity of this enzyme is increased during the initial stages of seizure activity. Similarly, if it is assumed that hexokinase activity is sufficient to phosphorylate glucose despite the increase in glycolysis which occurs during seizure activity and that phosphohexase isomerase activity is sufficient to allow for any changes in the concentration of the phosphorylated intermediates,[2,3] then one would not expect an appreciable change in the concentration of glucose-6-phosphate during seizure activity. However, the concentration of glucose-6-phosphate decreased during the seizures

which suggests that although initially the hexokinase activity may be sufficient, as the seizure progresses to the extensor tonic phase, hexokinase becomes the rate limiting factor. Somewhat similar conclusions have been reached by other investigators.[2,3]

The mechanism underlying the control of brain hexokinase activity is by no means certain. Glucose-6-phosphate inhibits hexokinase activity *in vitro*,[25] but other investigators have found that the concentration of this metabolite *in vivo* is too low to inhibit hexokinase activity.[2,26] Furthermore in the present investigation the concentration of glucose-6-phosphate was significantly reduced during the period when hexokinase activity was inhibited by leptazol.

The concentration of brain lactate rises shortly after the onset of the initial phase of the seizure irrespectively of how the seizure is caused. Since lactate is derived almost entirely from glucose metabolism, it would seem that the increase in glycolysis occurs very rapidly after the onset of increased neuronal activity. As no change in brain glucose is apparent at this time it is probable that the uptake of glucose into the brain and/or glycogenolysis is increased to meet the increased energy requirements.

The inhibition of hexokinase activity by leptazol does not appear to be due to the direct action of the drug on the enzyme as no effect could be detected *in vitro*. Furthermore anoxia, which is generally apparent during the terminal phases of the leptazol induced seizure, does not affect the activity of this enzyme. One possibility is that the activity of 6-phosphogluconate dehydrogenase is increased by the drug. Such an effect could reduce hexokinase activity by competing with hexokinase for the essential co-factor NADP.[28] However, no change in the activity of brain 6-phosphogluconate dehydrogenase could be found in rats which had been subjected to maximal leptazol seizures (unpublished).

It was found that addition of 2-mercaptoethanol to the buffer medium used to homogenize the brain after the rats had been treated with leptazol, abolished the inhibition of hexokinase activity. 2-Mercaptoethanol also increased the hexokinase activity of the control brain homogenate by approximately 2-fold. Other investigators have reported a similar effect of 2-mercaptoethanol on brain hexokinase activity.[12] However, it would not appear that this effect of 2-mercaptoethanol is due to its ability to block thiol groups as iodoacetate did not affect the hexokinase system under the same conditions. It is therefore not possible to draw any conclusion from the present investigation as to how leptazol stimulates and then inhibits brain hexokinase activity.

From these results it can be concluded that most of the changes in glycolysis are similar in convulsions initiated by three different types of seizure stimuli and represent an increase in the glycolytic flux. However, the changes in hexokinase activity induced by leptazol may reflect at least one major difference between the chemically and physically induced seizures.

REFERENCES

1. W. Gevers and H. A. Krebs, *Biochem. J.* **98,** 720 (1966).
2. O. H. Lowry and J. V. Passoneau, *J. biol. Chem.* **239,** 31 (1964).
3. O. H. Lowry, J. V. Passoneau, F. X. Hasselberger and D. W. Schulz, *J. biol. Chem.* **239,** 18 (1964).
4. B. Sacktor, J. E. Wilson and C. G. Tiekert, *J. biol. Chem.* **241,** 5071 (1966).
5. L. A. Woodbury and V. D. Davenport, *Archs int. Pharmacodyn. Ther.* **92,** 97 (1952).
6. W. E. Stone, *Biochem. J.* **32,** 1908 (1938).
7. A. Hugget, St. G. Nixon and D. A. Nixon, *Lancet* **ii,** 368 (1957).

8. K. F. GEY, *Biochem. J.* **64,** 145 (1956).
9. H. J. HOHORST, in *Methods in Enzymatic Analysis* (Ed. H. U. BERGMEYER), Academic Press, New York (1963).
10. E. C. SLATER, *Biochem. J.* **53,** 157 (1953).
11. R. SCHOLZ, H. SCHMITZ, T. BUCHER and J. D. LAMPEN, *Biochem. Z.* **331,** 71 (1959).
12. E. L. BENNET, J. B. DRORI, D. KRECH, M. R. ROSENWEIG and S. ABRAHAM, *J. biol. Chem.* **237,** 1758 (1962).
13. H. E. HIMWICH, in *Brain Metabolism and Cerebral Disorders.* Williams & Wilkins, Baltimore (1951).
14. W. E. STONE, J. E. WEBSTER and E. S. GUROJIAN, *J. Neurophysiol.* **8,** 233 (1945).
15. G. A. LE PAGE, *Am. J. Physiol.* **146,** 267 (1946).
16. J. R. KLEIN and N. S. OLSEN, *J. biol. Chem.* **167,** 747 (1947).
17. S. H. CARTER and W. E. STONE, *J. Neurochem.* **7,** 16 (1961).
18. F. M. MINARD, O. H. KANG and I. K. MUSHAHWAR, *J. Neurochem.* **12,** 279 (1965).
19. L. J. KING, O. H. LOWRY, J. V. PASSONEAU and V. VENSON, *J. Neurochem.* **14,** 549 (1967).
20. S. E. KERR and A. ANTAKI, *J. biol. Chem.* **122,** 49 (1937).
21. E. S. GURDJIAN, J. E. WEBSTER and W. E. STONE, *Res. Pub. Ann. Nerv. Ment. Dis.* **26,** 184 (1947).
22. S. M. GERSHOFF, G. W. NEWELL and W. E. STONE, *Archs Biochem. Biophys.* **21,** 74 (1949).
23. J. FOLBERGROVA, J. V. PASSONNEAU, D. H. LOWRY and J. SCHULZ, *J. Neurochem.* **16,** 191 (1969).
24. J. CROSSLAND and K. H. ROGERS, *Biochem. Pharmac.* **17,** 1637 (1968).
25. R. K. CRANE and A. SOLS, *J. biol. Chem.* **210,** 597 (1954).
26. K. UYEDA and E. RACKER, *J. biol. Chem.* **240,** 4682 and 4689 (1965).
27. S. R. NAHORSKI, D. H. ROBERTS and G. G. STEWART, *J. Neurochem.* **17,** 621 (1970).
28. B. E. LEONARD, unpublished.