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## Effect of experimentally induced seizures on some amino acids and ammonia in rat brain

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IN A PREVIOUS investigation, we studied the effects of electroconvulsive shock, leptazol and sound induced seizures on glucose metabolism in the rat brain.[1] The results of this study showed that there were differences between the changes associated with seizures induced by these different stimuli. It therefore seemed worthwhile to see if any differences occurred involving those amino acids which are metabolically connected with the citric acid cycle and which are also putative transmitter substances in the brain. Brain ammonia was also studied because there is evidence that this compound is a sensitive index of cerebral excitability.[2]

*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 in the previous study.[1]

The rats were killed by total immersion in liquid nitrogen. All determinations were made on the acid soluble extracts of whole brain. After centrifugation (800 *g* for 15 min), aliquots of the supernatant fraction were taken for the determination of ammonia,[3] glutamine,[4] glutamate,[5] tyrosine[6] and total free amino nitrogen.[7] $\gamma$-Amino-*n*-butyric acid ($\gamma$ABA) was estimated in the acidified ethanol extract of crushed frozen brain by paper chromatography.[8] Glutamate decarboxylase was estimated in homogenates of unfrozen brain by the monometric method of Roberts and Frankel.[9] In most cases the assays were made on brain extracts of animals killed during the full tonic phase of the seizure. A detailed description of the seizure phases induced by these stimuli is given in the previous publication.[1]

*Results and discussion*

Seizure activity induced by all three stimuli produced a rapid and marked elevation in the concentration of brain ammonia (Table 1). These increases occurred before the onset of the clonic phase of the seizure. Other investigators have shown that increased functional activity of the brain is associated with the accumulation of ammonia [10–12] but caution should be shown in assigning a role to ammonia in the initiation of seizure activity.[13]

The source of brain ammonia is unknown although there is some evidence that it is derived from cerebral proteins[14,15] or adenosine and glutamine.[16–18] In this study no change was found in the concentration of brain glutamine even during the tonic phase of the seizure. The concentration of brain glutamine in the untreated rats was 5·36 ± 0·087 μmoles/g wet weight.

TABLE 1. CHANGES IN THE CONCENTRATION OF BRAIN AMMONIA DURING SEIZURE ACTIVITY

| Control | Seizure phase: | Pre-clonic | Full tonic | Catatonic |
|---|---|---|---|---|
| | | Leptazol (100 mg/kg i.p.) | | |
| 0·077 ± 0·011 | | 0·128* ± 0·011 | 0·139* ± 0·033 | — |
| | | High intensity sound | | |
| | | 0·147* ± 0·009 | 0·146* ± 0·009 | 0·135† ± 0·011 |
| | | Electroshock | | |
| | | 0·143‡ ± 0·024 | 0·129‡ ± 0·016 | 0·169† ± 0·022 |

All results are expressed as μmoles/g wet weight of brain; each result represents the mean ± S.E.M. for at least six rats per group. The significance, as assessed by Students *t*-test, is shown by * $P < 0.001$. † $P < 0.01$. ‡ $P < 0.05$.

TABLE 2. CHANGES IN THE CONCENTRATION OF FREE AMINO NITROGEN DURING SEIZURE ACTIVITY

| Control | |
|---|---|
| | Leptazol |
| 13·3 ± 0·2 | 13·4 ± 0·6 |
| | High intensity sound |
| | 10·9* ± 0·1 |
| | Electroshock |
| | 12·9 ± 0·6 |

All results expressed as shown in Table 1. The experimental animals were killed during the full tonic phase of the seizure. Total free amino nitrogen was estimated as glutamate.

Free amino nitrogen could be another possible source of brain ammonia. However, a significant decrease only occurred during sound induced seizures (Table 2).

Glutamate did not change from that of the untreated group (4·33 ± 0·09 μmoles/g) even during the full tonic phase of the seizures. As the change in ammonia is approximately 80 nmoles/g, which is within the variation reported for the glutamate concentration, no conclusion can be drawn as to the contribution of this amino acid to the removal of ammonia from the brain. Nevertheless, others have suggested[1,9] that this amino acid does play a significant part in the removal of the excess ammonia during maximal seizure activity. Clearly a number of factors must contribute to the production and removal of ammonia during seizure activity but this study does not throw any light upon such mechanisms.

Of the other amino acids studied, tyrosine was found to increase during the full tonic phase of the seizures induced by all three stimuli (Table 3). One possible explanation for this increase is that it is a reflection of the anoxia which occurs during the terminal phase of the convulsion. This is supported by the finding of Tews and co-workers[20] who showed that brain tyrosine increases during anoxia. An accentuated turnover of brain proteins[21] may also contribute to the increase in brain tyrosine during seizure activity.

TABLE 3. CHANGES IN THE CONCENTRATION OF BRAIN TYROSINE DURING SEIZURE ACTIVITY

| Control | Leptazol | High intensity sound | Electroshock |
|---|---|---|---|
| 0·227 ± 0·002 | 0·330† ± 0·014 | 0·275‡ ± 0·007 | 0·392* ± 0·015 |

All results expressed as shown in Table 1. The experimental animals were killed during the full tonic phase of the seizure.

No change could be found in γABA during the maximal phase of the seizures irrespective of the method by which they were initiated. For the untreated rats, the brain γABA concentration was found to be 1·89 ± 0·039 μmoles/g. Such a finding is in agreement with those of other investigators.[22–24] Furthermore, no change could be detected in glutamate decarboxylase activity during the maximal seizure activity; for the control group the activity was found to be 207·8 ± 3 ml $CO_2$ liberated/g brain/hr. However Weichert and Gollnitz[25] found that a slight decrease (11–15 per cent) in the activity of this enzyme during the maximal phase of the seizure induced by these three stimuli. The reason for the discrepancy is not apparent, particularly as a manometric method for estimation of the enzyme was used in both instances.

From this study it would appear that the changes in ammonia and some amino acids which occur during the seizures induced by leptazol, high intensity sound and electroshock are similar. No evidence could be found that the seizures cause any change in the glutamine-glutamate-γABA pathway which some authors have suggested may be affected during convulsive activity.[19]

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## Influence of oxotremorine on glycogen content in various brain structures of rat brain

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THERE is much evidence to support the importance of corpus striatum in the genesis of static tremor.[1,2] It was shown that tremor-producing drug, oxotremorine (2′-oxo-1:4-dipyrrolidinobutyne) decreased the level of iron[3] and total flavines[4] in whole rat brain[5] and rat corpora striata.[6] Physostigmine and

TABLE 1. THE INFLUENCE OF ATROPINE AND PROPANOLOL ON GLYCOGENOLYTIC EFFECT OF OXO-TREMORINE

| Treatment of the animals | Brain structures | | | | |
|---|---|---|---|---|---|
| | Cortex cerebri | Caudate | Thalamus | Cortex cerebelli | Caudal brain stem |
| 1. Control | 52·2 ± 1·6 | 70·0 ± 1·7 | 42·3 ± 1·9 | 87·6 ± 3·7 | 102 ± 4·4 |
| 2. Oxotremorine (0·25 mg/kg) | 48·0 ± 2·6 | 20·2 ± 2·2* | 19·8 ± 1·1* | 83·6 ± 1·9 | 23·5 ± 3·0 |
| 3. Atropine (0·5 mg/kg) | 53·0 ± 1·4 | 72·4 ± 1·8 | 45·1 ± 1·3 | 81·0 ± 1·1 | 93·3 ± 3·7 |
| 4. Propranolol (10 mg/kg) | 56·8 ± 1·4 | 68·5 ± 1·6 | 38·9 ± 1·3 | 88·4 ± 2·2 | 108·6 ± 2·0 |
| 5. Atropine (0·5 mg/kg) +Oxotremorein (0·25 mg/kg) | 53·0 ± 1·8 | 74·0 ± 1·6 | 42·1 ± 1·6 | 93·4 ± 2·2 | 98·5 ± 2·4 |
| 6. Propranolol (10 mg/kg) +Oxotremorine (0·25 mg/kg) | 49·8 ± 1·5 | 71·4 ± 1·7 | 45·6 ± 1·4 | 95·4 ± 2·5 | 101 ± 2·6 |

* $P < 0.01$ in comparison with the controls.

The content of glycogen is expressed in mg% of freshly frozen tissue. The numbers indicate the mean value (M) of five experiments ± S.E.M.