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THE EFFECT OF AUDIOGENIC SEIZURES ON LABILE NITROGEN AND PHOSPHORUS CONTAINING COMPOUNDS IN THE RAT BRAIN

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Abstract—The brain lactate and ammonia levels increased and ATP, creatine phosphate and hexose phosphate levels decreased in a susceptible strain of rats when they were subjected to a prolonged auditory stimulus. The maximum changes in these compounds occurred during the catatonic phase of the seizure. There was a significant negative correlation between the rise in brain ammonia and fall in ATP. The possible sources of the endogenous brain ammonia formed during the seizure are discussed.

A significant fall in adrenal ascorbic acid only occurred during the catatonic phase of the seizure.

SOME strains of rats have convulsions when subjected to high intensity sound.[1, 2] Kolaušek *et al.*[1] studied the nitrogen metabolism in the brains of a strain of rats that were susceptible to audiogenic seizures and found that they contained more free amide nitrogen and less free ammonia nitrogen than a non-susceptible strain. The present study was undertaken to investigate changes in labile nitrogen and phosphorus compounds in another susceptible strain during the different phases of the audiogenic seizure.

METHODS

Female rats (50–60 g) were used. These were originally of the Wistar strain but have been inbred for many generations. Animals which had convulsions when subjected to high intensity sound, produced by an electric door bell, for 2 min were killed during the wild running, tonic and catatonic phases of the seizure by rapid total immersion in liquid oxygen. Rats which did not convulse when subjected to the sound stimulus were killed in the same way. The control group consisted of rats of the same strain which were not subjected to the sound stimulus. The brains were rapidly removed while still frozen, weighed and homogenized in 10% (v/v) trichloracetic acid (TCA). After centrifuging at approximately 3,500 rev/min for 5 min the clear supernatant was removed and assayed for ammonia,[3] free amino nitrogen,[4] lactic acid,[5] creatine phosphate,[6] ATP,[7] inorganic phosphate,[7] and alkaline soluble phosphates[8] (mainly hexose phosphates). Glutamine was estimated by the method of Harris[9] following the hydrolysis of a portion of the TCA extract. The residue remaining after the removal of the supernatant was dissolved in hot 30% (w/v) KOH and the 'bound' glycogen estimated by the method of Russel and Bloom.[10] The adrenal glands were removed after the carcasses had thawed. The adrenals were then weighed, homogenized in 6% (v/v) TCA and the ascorbic acid estimated.[11]

RESULTS

Approximately 25 per cent of the rats had convulsions when subjected to the auditory stimulus for 2 min. The time of onset of the seizure phases is given in Table 1.

TABLE 1. TIME OF ONSET OF THE SEIZURE PHASES

Seizure phase	Time (sec)
Wild running	34·7 ± 2·2* (13)
Tonus	82·5 ± 2·3 (8)
Catatonia	91·9 ± 2·0 (10)

* Mean ± s.e.m.
Number of animals per group in parenthesis.

The "wild running" phase of the seizure was generally followed by a short period when the rat was inactive. If the auditory stimulus was discontinued at this stage then the animals recovered without passing through any further phases of the seizure. If however the auditory stimulus was continued then the period of inactivity was followed by the rat running rapidly around the cage and eventually having tonic convulsions or, more generally, going into catatonia. The rat recovered from catatonia after 5–10 min but was behaviourly depressed for several hours.

The results of the chemical determinations show that the maximum changes occurred during the catatonic phase of the seizure (Fig. 1). No observable changes occurred in rats that were subjected to the auditory stimulus but which did not convulse. There was a statistically significant rise in brain ammonia during all phases of the seizure and it was 50 per cent above the control value during the catatonic phase. It seems unlikely that this increase in brain ammonia was derived primarily from the free amino nitrogen pool because the slight decrease in total free amino nitrogen only occurred during the tonic phase of the seizure and returned to the control level during the catatonic phase. The level of brain glutamine was unchanged during the seizure. Creatine phosphate, hexose phosphates and ATP levels were considerably reduced during the seizure. The creatine phosphate and hexose phosphate concentration decreased in all phases of the seizure and was 22 and 43 per cent respectively below the control value during the catatonic phase. However the ATP level only decreased during the tonic and catatonic phases and was then approximately 33 per cent below the control value. There was a significant negative correlation between the rise in brain ammonia and the fall in ATP ($r = -0{\cdot}44$, $t = 2{\cdot}43$, $P < 0{\cdot}02 > 0{\cdot}01$). The brain lactate concentration increased during the catatonic phase to a level approximately 25 per cent above the control value but the concentration of 'bound' glycogen was unchanged. A significant depletion of adrenal ascorbic acid was only detected during the catatonic phase of the seizure (Fig. 2).

DISCUSSION

Bentley[1] found that 40–60 per cent of the Nicolas strain of rats used by him developed seizures when subjected to high intensity sound and moreover most of the susceptible rats showed all phases of the seizure. In contrast only approximately 25 per cent of the

Wistar sub-strain were susceptible and of those approximately one third did not show the complete seizure pattern. Besides the genetical differences other factors such as the nature of the auditory stimulus, the age and the sex of the rats[12] may be

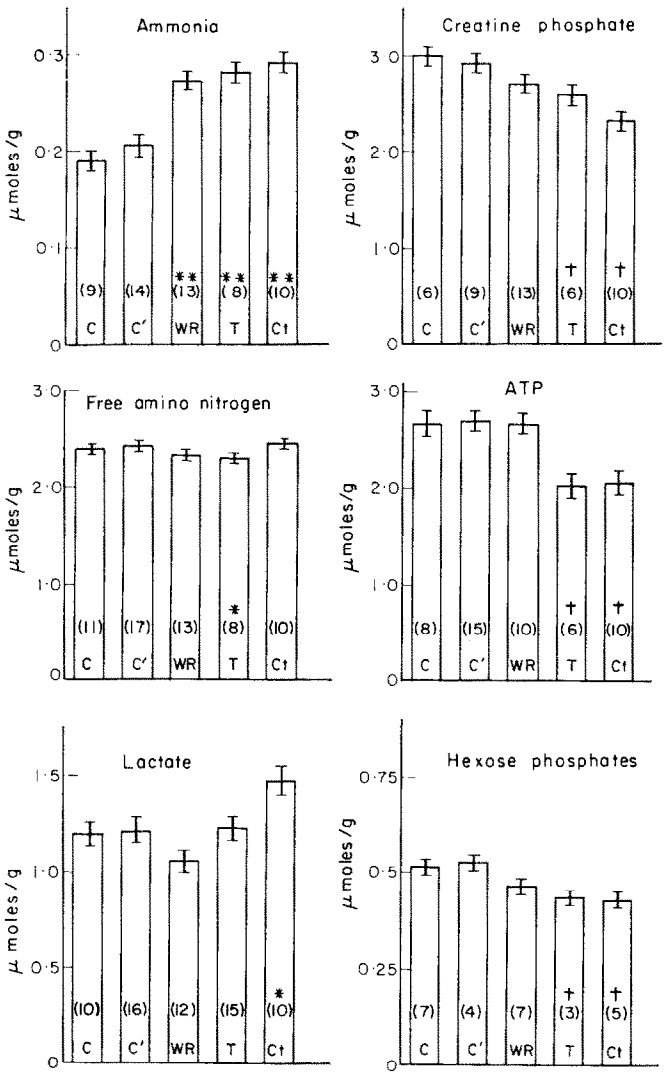

FIG. 1. Changes in labile compounds in the rat brain during audiogenic seizures.
C: controls rats killed in liquid oxygen without first being exposed to the sound stimulus.
C[1]: control rats killed after exposure to the stimulus for 2 min.
WR, T and Ct: rats killed during the wild running, tonic and catatonic phases of the seizure respectively.

Number of animals in each group indicated in the brackets. Results, in μmoles/g wet weight of brain, expressed as the mean ± standard error. Significance of difference between control and experimental groups expressed by $^{**}P < 0{\cdot}001$; $^{*}P < 0{\cdot}02 > 0{\cdot}01$; $\dagger P < 0{\cdot}05$.

Free amino nitrogen and hexose phosphates expressed as glutamic acid and glucose 6-phosphate respectively.

The following compounds were unchanged: glutamine, 'bound' glycogen and inorganic phosphate.

important in explaining the difference in seizure susceptibility of these strains. However it is not unreasonable to assume that the changes occurring during the different phases of the seizure are qualitatively, if not quantitatively, similar in most strains of susceptible rats.

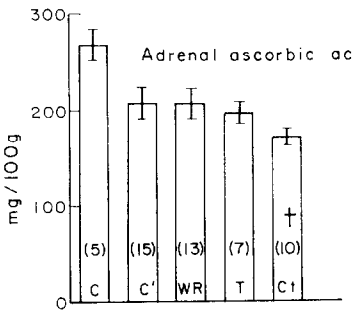

FIG. 2. Changes in adrenal ascorbic acid during audiogenic seizures.
Details as for Fig. 1. Results expressed as mg. ascorbic acid/100 g wet weight of tissue. †P < 0·05 > 0·02.

The most significant change occurring in the brains of rats during audiogenic seizures is the rise in ammonia and fall in high energy phosphate compounds. The process of endogenous ammonia formation in the brain is complex and the exact mechanism is not well understood. Nevertheless several suggestions have been made as to the immediate source of ammonia resulting from an increased excitability of the central nervous system. Weil-Malherbe and Green[13] and Vrba[14] found evidence for the view that brain ammonia was derived from the amide groups of brain proteins whilst others[15] have suggested that adenylic acid and glutamine are the main sources. It seems unlikely that the ammonia formed during audiogenic seizures is derived from brain glutamine as the concentration of this amide was unchanged during the seizure. However, there was a significant fall in free amino nitrogen during the tonic phase of the seizure which could be a source of ammonia if only a minor one.

There are two other possible ways in which the increase in the concentration of ammonia in the brain could be explained. The negative correlation between the rise in ammonia and fall in ATP suggests that ammonia may be derived by an endergonic process from otherwise stable nitrogen sources. Alternatively there may be a failure in the mechanism whereby endogenous ammonia is normally removed from the brain. The main mechanism for the detoxification of ammonia in the brain is probably via the system:

$$\alpha\text{-ketoglutarate} \overset{NH_3}{\rightleftharpoons} \text{glutamic acid} \overset{NH_3}{\rightleftharpoons} \text{glutamine}^{16}$$

The terminal stage of this reaction leading to the formation of glutamine requires 1 mole ATP per mole of ammonia.[17] As convulsions induced by any means (by chemical, by electrical or by an audiogenic stimulus) cause a decrease in the high energy phosphate compounds in the brain, the rise in brain ammonia during audiogenic seizures may be due to an insufficient amount of ATP to 'bind' the ammonia *via* the α-ketoglutarate–glutamine system. It is difficult to decide on the basis of the

results obtained, which of these possibilities could account for the observed changes in brain ammonia and labile phosphate compounds. It is evident however that a failure in the detoxification mechanism cannot account for all of the additional ammonia formed during the seizures because according to this mechanism a much lower concentration of ATP would be anticipated than was actually found before there would also be an appreciable rise in ammonia. Therefore it seems likely that several factors are involved in the increased formation of brain ammonia to which the labile nitrogenous compounds determined in these experiments contribute only a minor part.

The rise in brain lactate during the catatonic phase of the seizure is probably the result of a generalized increase in carbohydrate metabolism. This is further suggested by the fall in hexose phosphates during the seizure. However the rise in brain lactate may be secondary to the rise in ammonia as has been suggested by the results of several investigators[18–20] who found that ammonium ions stimulate aerobic glycolysis in brain tissue *in vitro*. The concentration of ammonium ions required to produce this effect *in vitro* was at least twice the ammonia concentration found *in vivo* in the present experiments[18] which suggests that the rise in ammonia may be making only a minor contribution to the rise in brain lactate at least in these experiments.

There was no significant change in 'bound' glycogen during the seizure. This is not surprising since it has been shown[10, 21, 22] that only the acid extractable tissue glycogen can be readily metabolized during short periods of increased excitability whereas the residual fraction is relatively stable.

Adrenal ascorbic acid was used as an index of the intensity and duration of physiological stress.[23] The control values for adrenal ascorbic acid reported here are lower than those reported in the literature.[24, 25] This is probably due to the delay in removing the adrenals from the animals following death as estimations of the adrenal ascorbic acid in this strain of animals when killed by decapitation and assayed immediately give values similar to those reported in the literature.[26] In the present experiments a significant decrease in the adrenal ascorbic acid content only occurred during the catatonic phase of the seizure.

In conclusion, these results suggest that the maximal effect of the audiogenic seizure, as measured by the changes in some labile nitrogen and phosphorus compounds and lactic acid in the brain, is found during the catatonic phase of the seizure. It seems unlikely that the depletion of adrenal ascorbic acid is a direct reflection of the intensity of the catatonic phase of the seizure as the depletion takes several minutes to develop irrespective of the nature or duration of the stimulus.

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