DYNAMICS OF AMMONIA FORMATION AND FIXATION IN RAT BRAIN TISSUES DURING EPILEPTOGENIC ACOUSTIC STIMULATION

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At the end of phase I of motor excitation produced by very strong acoustic stimulation the content of ammonia, glutamin, and glutamic acid in the cerebral cortex, medulla, and cerebellum falls. Transient inhibition of motor activity is observed at this period. In phase II of motor excitation the concentration of preformed ammonia in the cerebral cortex increases in the mesencephalon, medulla, and cerebellum, while the concentration of glutamine and glutamic acid in these parts of the brain falls considerably. It is considered that changes in the ammonia concentration in the brain are responsible for the concept of clonic convulsions.

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The relationship between ammonia formation and fixation in nerve tissue and its functional state has often been investigated [1, 2, 6]. It has been shown conclusively that predominance of excitation in nerve tissue is associated with an increase in the concentration of preformed ammonia. In the period of excitation the rate of ammonia formation is much higher than the rate of its fixation. According to modern views of the functional biochemistry of the brain, therefore, the ammonia level in a particular part of the brain indicates the state of fundamental nervous processes or, more exactly, the predominance of one over the other.

Our previous investigations [5, 7] showed that an epileptogenic discharge is characterized by a sharp rise in the free ammonia concentration. It has also been shown [8, 9] that ammonium salts can produce epileptiform convulsions in which ammonia acts as a toxic epileptogenic agent.

In this investigation we studied the liberation and fixation of ammonia (by determining the levels of preformed ammonia, glutamic acid, and glutamine, and the amide nitrogen of total proteins in various parts of the brain) in rats exposed to very strong epileptogenic acoustic stimulation.

EXPERIMENTAL METHOD

Experiments were carried out on rats, mostly males, in a specially fitted chamber.

The rats were frozen in liquid oxygen in a waking state (control) in a state of motor excitation (at the end of phases I [4] and II of motor excitation before falling into a stupor), in the middle of the phase of clonic convulsions, and at the end of the phase of clonic convulsions before the onset of coma.

The level of free (preformed) ammonia, glutamic acid, and glutamine was determined in the cerebral cortex, diencephalon, mesencephalon, pons, medulla, and cerebellum, and the contents of amide nitrogen (nitrogen of labile and firmly bound amide groups) in total proteins was determined in the cerebral cortex, brain stem, and cerebellum. Preformed ammonia was estimated by a microdiffusion method [10] and glutamine and glutamic acids by paper chromatography; amide groups of total proteins split during acid hydrolysis for 10 min and 2 h were investigated [3].

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TABLE 1. Content of Free Ammonia (M ± m) in Different Parts of Rat Brain during Convulsions Caused by Acoustic Stimulation (Results of 10 Experiments)

Series of experiment	Free ammonia (in mg %) in fresh tissue					
	cortex	dienceph-alon	mesenceph-alon	pons	medulla	cerebellum
Control	0.34 ± 0.02	0.51 ± 0.08	0.66 ± 0.02	0.53 ± 0.01	0.72 ± 0.06	0.54 ± 0.00
End of phase I of motor excitation	0.23 ± 0.02 P < 0.001	0.43 ± 0.05 P < 0.5	0.71 ± 0.10 P > 0.5	0.43 ± 0.07 P < 0.2	0.49 ± 0.06 P < 0.02	0.38 ± 0.06 P > 0.02
End of phase II of motor excitation	0.41 ± 0.05 P < 0.2	0.40 ± 0.06 P < 0.2	0.83 ± 0.06 P < 0.02	0.54 ± 0.11 P > 0.5	0.90 ± 0.16 P < 0.5	0.49 ± 0.09 P > 0.5
Clonic phase	0.63 ± 0.04 P < 0.001	0.41 ± 0.05 P < 0.5	0.56 ± 0.03 P < 0.02	0.71 ± 0.12 P < 0.2	0.51 ± 0.06 P < 0.05	0.32 ± 0.02 P < 0.001
End of tonic phase	0.33 ± 0.03 P < 0.5	0.48 ± 0.0 P > 0.5	0.61 ± 0.11 P > 0.5	0.59 ± 0.09 P > 0.5	0.67 ± 0.08 P > 0.5	0.49 ± 0.10 P > 0.5

TABLE 2. Content of Glutamine (M ± m) in Different Parts of Rat Brain during Convulsions Produced by Acoustic Stimulation (Results of 5 Experiments)

Series of experiment	Glutamine (in mg %) in fresh tissue					
	cortex	dienceph-alon	mesenceph-alon	pons	medulla	cerebellum
Control	93.0 ± 1.17	80.3 ± 0.95	73.9 ± 1.98	68.0 ± 4.66	70.9 ± 3.65	107.7 ± 3.97
End of phase I of motor excitation	64.1 ± 4.36 P < 0.001	55.0 ± 2.37 P < 0.001	46.8 ± 2.88 P < 0.001	37.3 ± 1.87 P < 0.001	42.9 ± 2.71 P < 0.001	61.8 ± 2.67 P < 0.001
End of phase II of motor excitation	48.8 ± 1.93 P < 0.001	47.3 ± 1.94 P < 0.001	36.6 ± 1.64 P < 0.001	22.2 ± 2.71 P < 0.001	26.6 ± 0.90 P < 0.001	46.9 ± 2.48 P < 0.001
Clonic phase	74.8 ± 1.81 P < 0.001	78.1 ± 0.53 P < 0.1	49.9 ± 0.91 P < 0.001	46.9 ± 2.40 P < 0.01	51.8 ± 5.38 P < 0.02	77.6 ± 1.48 P < 0.001
End of tonic phase	82.9 ± 1.82 P < 0.002	87 ± 3.00 P < 0.05	65.7 ± 1.46 P = 0.01	63.2 ± 1.57 P < 0.5	58.7 ± 2.37 0.02 < P < 0.05	103.0 ± 1.83 P < 0.5

TABLE 3. Content of Glutamic Acid (M ± m) in Different Parts of Rat Brain during Phases of Convulsion Produced by Acoustic Stimulation (Results of 5 Experiments)

Series of experiment	Glutamic acid (in mg %) in fresh tissue					
	cortex	dienceph-alon	mesenceph-alon	pons	medulla	cerebellum
Control	193.1 ± 3.11	149.6 ± 1.92	123.4 ± 6.63	114.0 ± 6.1	131.9 ± 9.0	152.5 ± 6.86
End of phase I of motor excitation	171.4 ± 2.69 P < 0.001	132.3 ± 5.74 0.02<P<0.05	88.7 ± 6.45 P < 0.01	64.6 ± 1.75 P < 0.001	100.0 ± 1.85 P < 0.01	154.0 ± 3.27 P > 0.5
End of phase II of motor excitation	141.3 ± 4.70 P < 0.001	127.0 ± 5.00 P < 0.01	90.7 ± 2.53 P < 0.002	79.0 ± 2.85 P < 0.001	72.6 ± 1.38 P < 0.001	123.8 ± 3.80 P < 0.01
Clonic phase	151.6 ± 3.00 P < 0.001	139.6 ± 5.53 P < 0.25	93.0 ± 2.21 P < 0.01	88.1 ± 1.86 P < 0.01	78.3 ± 3.00 P < 0.001	108.0 ± 2.91 P < 0.001
End of tonic phase	147.8 ± 3.00 P < 0.001	157.1 ± 2.34 0.02<P<0.05	98.0 ± 2.34 P < 0.01	95.0 ± 1.32 P < 0.02	97.4 ± 1.67 P < 0.01	99.0 ± 1.15 P < 0.001

TABLE 4. Amide Groups of Total Proteins (M ± m) in Different Parts of Rat Brain during Phases of Convulsion Produced by Acoustic Stimulation (Results of 7 Experiments)

Series of experiment	Amide groups of total proteins (in g amide nitrogen/mg dry protein)					
	labile			firmly bound		
	cortex	brain stem	cerebellum	cortex	brain stem	cerebellum
Control	2.54 ± 0.10	1.97 ± 0.13	3.20 ± 0.20	3.80 ± 0.20	3.60 ± 0.12	4.20 ± 0.43
End of phase I of motor excitation	1.94 ± 0.30 P > 0.05	1.67 ± 0.04 P < 0.05	1.94 ± 0.23 P < 0.002	3.54 ± 0.17 P < 0.5	2.84± 0.12 P < 0.001	3.20 ± 0.26 P > 0.05
End of phase II of motor excitation	0.80 ± 0.09 P < 0.001	0.60 ± 0.04 P < 0.001	1.45 ± 0.14 P < 0.001	4.20 ± 0.19 P < 0.25	3.80 ± 0.37 P > 0.5	2.50 ± 0.18 P < 0.01
Clonic phase	3.00 ± 0.14 P < 0.02	2.25 ± 0.22 P < 0.5	3.94 ± 0.35 P < 0.1	2.74 ± 0.21 P < 0.01	3.70 ± 0.37 P > 0.5	3.30 ± 0.34 P < 0.25
End of tonic phase	3.93 ± 0.37 P < 0.01	3.35 ± 0.27 P < 0.001	6.70 ± 0.39 P < 0.001	5.34 ± 0.65 P < 0.05	4.90± 0.50 0.02<P<0.05	6.40 ± 0.37 P = 0.002

EXPERIMENTAL RESULTS

The results showed that at the end of phase I of motor excitation, when the animals usually lie still for some time and do not respond to acoustic stimulation, there was a statistically significant decrease in the ammonia concentration (Table 1) in the cerebral cortex, medulla, and cerebellum, together with a marked decrease in the concentrations of glutamine (Table 2) and glutamic acid (Table 3) in all parts of the brain investigated except the cerebellum, where the glutamic acid level was unchanged. No changes were found at this time in the amide nitrogen of the total proteins in the cortex; the level of labile and firmly bound amide groups in the brain stem was lowered, while in the proteins of the cerebellum the concentration of labile amide groups was lowered and that of firmly bound amide groups unchanged (Table 4).

It can thus be concluded from the results described above that at the end of phase I of motor excitation inhibition is dominant in the central nervous system of the animals, as a result of which the animal does not respond for some time (8-12 sec) to the continued action of the acoustic stimulus.

The next phase (II) of motor excitation is characterized by elevation of the preformed ammonia level in the cerebral cortex, mesencephalon, medulla, and cerebellum and by a considerable decrease in the concentration of glutamic acid and glutamine in all parts of the brain studied. The level of labile amide groups in proteins of the cerebral cortex, brain stem, and cerebellum falls sharply, while a decrease in the firmly bound amide groups is observed only in total proteins of the cerebellum.

Consequently, the preconvulsive state is characterized biochemically by predominance of excitation, particularly marked in the higher levels of the central nervous system, which are evidently responsible for the generation of clonic convulsions. In the clonic phase, for instance, a further increase in the ammonia concentration in the cerebral cortex was found (by 85.3% compared with the control, whereas at lower levels the concentration of preformed ammonia was essentially unchanged or actually lowered (mesencephalon, medulla, cerebellum). In this phase of the convulsion the glutamine concentration in the brain was increased, while the glutamic acid concentration remained as before. Characteristically in the clonic phase of the convulsions the greatest changes in amide nitrogen were observed in the cortex, while in the brain stem and cerebellum no such changes could be detected.

It can thus be concluded from these investigations that preformed ammonia, as it accumulates in the motor cortex as a result of excessive motor excitation caused by acoustic stimulation, can produce convulsions. It may be suggested that preformed ammonia, as an epileptogenic agent, produces a paroxysmal discharge in the motor cortex and this gives rise to clonic convulsions. This agrees with the views expressed by most workers on the genesis of the clonic phase of epileptiform and epileptic convulsions.

In the phase of tonic convulsions the concentration of preformed ammonia is considerably increased in the medulla, and this can be associated with epileptogenic discharges taking place in this region, while in the mesencephalon and diencephalon no significant changes compared with the control are observed. At

the end of the tonic phase, however, amidation of proteins was found in all parts of the brain investigated. It can be postulated that the mechanism of formation of the tonic phase of an acoustic convulsion is analogous to the physiological mechanisms of its formation in other experimentally produced epileptiform convulsions. According to the results of biochemical and electrophysiological investigations [7, 8], the tonic phase is due to epileptogenic discharges in the lower parts of the brain stem, and the results of the present investigation support this view.

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