METRAZOL KINDLING IN RATS DIFFERING IN RESISTANCE TO HYPOXIA

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UDC 615.213.015.4.076.9

KEY WORDS: kindling; hypoxia; correlation; metrazol

Pharmacological kindling, produced by daily administration of epileptogens in subconvulsive doses, increases the predisposition of animals to seizures and culminates in the onset of seizures in response to injection of the same subconvulsive dose of the epileptogen [7, 10, 12, 15]. The development of this effect depends on the individual sensitivity of the animals to the action of convulsants. It has been shown on a model of focal epilepsy that the resistance of animals to epileptogens correlates with their resistance to hypoxia [1].

In the investigation described below correlation was discovered between individual sensitivity to hypoxia and the degree of development of predisposition to seizures during pharmacological kindling induced by metrazol.

EXPERIMENTAL METHOD

Resistance to hypoxia was determined in 60 noninbred male albino rats weighing 200-250 g in a pressure chamber, in which the atmospheric pressure was lowered to a level corresponding to an altitude of 11,000 m. The following parameters were recorded: the duration of loss of posture, the duration of reversible respiratory arrest (conventionally known as the "life time"), and the time of recovery of posture. On the basis of these parameters 15 animals with low resistance to hypoxia (LRA) and 15 animals with high resistance (HRA) were selected. One week after exposure to hypoxia the selected rats were given an injection of metrazol (25 mg/kg, intraperitoneally) daily between 4 and 6 p.m. During 1 h after injection of metrazol, the character and severity of the seizure reactions were observed. The intensity of the seizures was expressed in points, on the following scale: 0) seizures absent, 1 point) myoclonic spasms of the head and trunk, 2 points) clonic convulsions of the forelimbs, 3 points) standing up on the hind limbs ("kangaroo" posture) or repeated clonic convulsions, 4 points) clonic convulsions with the animal falling on its side, 5 points) lethal convulsions or repeated episodes of falling on the side. Each day the average score in each group was determined in animals with seizure responses.

EXPERIMENTAL RESULTS

The data reflecting resistance of LRA and HRA to hypoxia are given in Fig. 1. They show significant differences in the resistance of the respiratory center between these two groups of animals, mainly with respect to parameters characterizing resistance to hypoxia. The average life time of the LRA was 3.94 ± 0.25 min, of the HRA 11.5 ± 0.56 min.

The results of an experiment to study the kindling effect of daily injections of a subthreshold dose of metrazol into LRA and HRA are given in Tables 1 and 2. They indicate that metrazol kindling develops differently in LRA and HRA. In the LRA group, seizure reactions with an intensity of 4.0 ± 0.22 points occurred in 14 of 15 rats, whereas in the HRA group they occurred in only eight of 15 rats; the intensity of the seizures in this group, moreover, was 2.75 ± 0.39 points. The increase in the intensity of the seizure reactions took place much more rapidly in the LRA than in the HRA: for

Laboratory of General Pathology of the Nervous System, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. Department of Normal Physiology, Patrice Lumumba Peoples' Friendship University, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 240-243, September, 1991. Original article submitted February 11, 1991.

 0007-4888/91/0009-1232$12.50

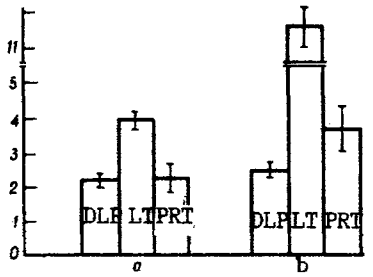

Fig. 1. Resistance of LRA (a) and HRA (b) to hypoxia. Abscissa, parameter of hypoxic resistance: DLP) duration of loss of posture, LT) life time at "altitude" of 11,000 m, PRT) posture recovery time; ordinate, time (in min).

TABLE 1. Effect of Daily Injections of Metrazol (25 mg/kg) on Intensity of Seizure Reaction in LRA

Day of experiment	Total number of animals	Number of animals with seizures	Intensity of seizure reaction, points					Average severity of reaction, points
			1	2	3	4	5	
1	15	—	—	—	—	—	—	0
2	15	2	2	—	—	—	—	1
3	15	2	1	1	—	—	—	1,5±0,5
4	15	4	—	3	1	—	—	1,5±0,56
5	15	4	—	3	1	—	—	1,5±0,56
6	15	4	—	3	1	—	—	1,5±0,56
7	15	5	1	3	1	—	—	2,0±0,35
8	15	5	—	4	1	—	—	2,2±0,22
9	15	8	3	2	3	—	—	2,0±0,35
10	15	9	3	3	1	2	—	2,2±0,42
11	15	9	3	2	2	2	—	2,3±0,43
12	15	10	4	2	2	2	—	2,2±0,41
13	15	10	4	2	2	2	—	2,2±0,41
14	15	11	5	1	3	2	—	2,2±0,4
15	15	12	4	3	3	2	—	2,25±0,34
16	15	13	5	3	3	2	—	2,15±0,33
17	15	13	3	3	5	2	—	2,46±0,3
18	15	14	3	4	2	5	—	2,64±0,34
19	15	14	3	3	2	5	1	2,86±0,39
20	15	14	—	5	2	6	1	3,21±0,29
21	14	13	—	3	3	7	—	3,31±0,25
22	14	13	—	2	2	8	1	3,61±0,25
23	13	12	—	—	3	6	3	4,0±0,22

seizures with an intensity of 2-2.5 points to develop in LRA 7-17 injections were needed, compared with 20-26 injections for HRA. Generalized clonicotonic convulsions with the animal falling on to its side, and with death of the rats (5 points) occurred in five LRA after 21-23 injections, whereas in the HRA group convulsions of the same intensity did not arise even after 27 injections of metrazol.

Significant differences in the intensity of the seizure reactions in LRA and HRA were found on the 17th day of stimulation: in LRA the intensity of the seizures by this time was 2.46 ± 0.3 points, compared with 1.2 ± 0.22 points ($p < 0.05$) in HRA. On the 23rd day of injection of metrazol, the manifestations of seizures reached 4.0 ± 0.22 points in LRA but only 2.28 ± 0.45 points in HRA ($p < 0.01$).

Differences in the character of the behavioral disturbances also were found between LRA and HRA. From 3 to 5 min after the fourth or fifth injection of metrazol a decrease of mobility was observed: periods of locomotor activity were interrupted by brief (3-6 sec) episodes of complete immobility, which occurred with a frequency of 2-4 times per minute. The rats also displayed increased anxiety, as shown by frequent washing and defecation; the rats constantly stood up on their hind paws and sniffed the floor and walls of the chamber. After the fourth to the ninth injections these animals developed tremor of the head, and twitching of the snout and ears. Before the ninth injection, LRA exhibited passive-defensive reactions: running away, and jumping during any attempt to handle the rats. Subsequent injections of metrazol (10th-17th injections) induced myoclonic spasms of the head and myoclonic convulsions of the forelimbs in these animals.

TABLE 2. Effect of Daily Injection of Metrazol (25 mg/kg) on Intensity of Seizure Reaction in HRA

Day of experiment	Total number of animals	Number of animals with seizures	Intensity of seizure reaction, points					Average severity of reaction, points
			1	2	3	4	5	
1	15	—	—	—	—	—	—	0
2	15	1	1	—	—	—	—	1
3	15	2	2	—	—	—	—	1
4	15	2	2	—	—	—	—	1
5	15	2	2	—	—	—	—	1
6	15	3	3	—	—	—	—	1
7	15	3	3	—	—	—	—	1
8	15	3	3	—	—	—	—	1
9	15	3	3	—	—	—	—	1
10	15	3	3	—	—	—	—	1
11	15	3	3	—	—	—	—	1
12	15	3	3	—	—	—	—	1
13	15	3	3	—	—	—	—	1
14	15	4	4	—	—	—	—	1
15	15	4	4	—	—	—	—	1
16	15	4	4	—	—	—	—	1
17	15	5	4	1	—	—	—	1,2±0,22
18	15	5	4	1	—	—	—	1,2±0,22
19	15	6	4	2	—	—	—	1,3±0,23
20	15	6	4	1	1	—	—	1,5±0,37
21	15	6	3	1	2	—	—	1,83±0,44
22	15	7	3	1	3	—	—	2,0±0,41
23	15	7	2	2	2	1	—	2,28±0,45
24	15	8	3	2	2	1	—	1,05±0,43
25	15	8	2	2	3	1	—	2,37±0,4
26	15	8	1	2	4	1	—	2,62±0,35
27	15	8	1	2	3	2	—	2,75±0,39

During this period the passive-defensive reactions began to be transformed into active-defensive: on an attempt to handle the rats they stood up on their hind limbs and tried to bite, and fights between the animals frequently took place in the cages. After 17-23 injections manifestations of the seizure syndrome rapidly progressed in the LRA: episodes of clonic convulsions of the forelimbs changed into generalized clonicotonic convulsions of the whole body, with the animal falling on to its side. The duration of the generalized convulsions varied in different animals from 10 to 300 sec. At the end of the episode, postictal depression developed. In most (12) rats repeated convulsions were observed, and in eight of the animals this progressed into status epilepticus, ending with death of five rats. During this period the aggressiveness of the rats increased sharply, they fought one another continually, stood on their hind limbs for a long time, and split up into pairs, crying, scratching, and biting each other, their snouts stained with blood.

In the HRA the first injections of metrazol (1st-5th) caused no disturbances of behavior. Only after the 6th-11th injections did these animals develop passive-defensive reactions, periods of complete locomotor immobility, and signs of anxiety. After the 12th-16th injections, with each fresh injection the HRA showed increased anxiety and gave stronger passive defensive reactions; however, no seizures were observed. Only after the 17th-27th injections did half of the HRA (eight rats) begin to exhibit manifestations of seizures, which gradually increased in intensity: tremor and myoclonic spasms of the head, clonic convulsions of the forelimbs and trunk, but no visible generalized convulsions or falling on to the side. In HRA passive defensive behavior was replaced by active defensive behavior only extremely rarely, and only in those rats in which the most marked convulsive reactions were observed.

Repeated injections of subconvulsive doses of metrazol into rats thus lead to the appearance not of one, but of three epileptic syndromes: akinetic ("freezing" with immobility), emotional-behavioral (anxiety, passive- and active-defensive reactions), and convulsive. This effect was observed also in picrotoxin-induced kindling [8]. According to the general theory of generator, systemic, and determinant mechanisms of neuropathological syndromes [6], each syndrome has its own pathological system with an initial pathological determinant. The severity of the above-mentioned syndromes depended both on the number of injections and on the individual reactivity of the animals, which correlates with resistance of the animals to hypoxia. In the LRA increased predispostion to seizures develops more rapidly until the appearance of convulsive reactions characteristic of the clinical picture of a major epileptic fit, whereas in HRA this syndrome is formed much more slowly, and in addition, the convulsive reactions are weaker and more characteristic of a minor epileptic fit. These observations are in agreement with the results of an investigation which showed that rats with high resistance to hypoxia are much more resistant to an electroconvulsive fit than LRA [3]. The akinetic syndrome appeared in HRA after a larger

number of metrazol injections than with LRA, but no difference could be found in the character or intensity of this syndrome. Some degree of correlation was observed between the severity and character of the emotional-behavioral disturbances and the intensity of the developing convulsive syndrome: in HRA mainly passive-defensive reactions developed, whereas in LRA they were transformed into active-defensive. Kindling raised the level of anxiety of the HRA much more slowly than LRA. Animals with a low level of anxiety are known to be more resistant to acute hypoxia [4].

The results of these experiments suggest that in HRA and LRA activity of structures of the proepileptic and/or antiepileptic systems are initially different [6, 14]. It can be tentatively suggested that activity of the antiepileptic system is depressed in LRA [6]. This suggestion is supported by data showing differences in the brain catecholamine levels in rats differing in their resistance to hypoxia: in HRA the catecholamine level is 2 or 3 times higher than in LRA, and adaptation to hypoxia is accompanied by a two-fourfold increase in the noradrenalin concentration in the cerebral cortex of LRA [5]. Considering that catecholamines have an inhibitory action on epileptic activity in the cerebral cortex [13], the protective action of such adaptation on seizure activity can be explained by an increase in their concentration during adaptation to hypoxia [2, 9, 11]. Adaptation to hypoxia probably also increases tonic activity of the antiepileptic system.

LITERATURE CITED

1. N. A. Agadzhanyan and V. I. Torshin, Byull. Éksp. Biol. Med., No. 6, 20 (1983).
2. N. A. Agadzhanyan and V. I. Torshin, Byull. Éksp. Biol. Med., No. 12, 683 (1986).
3. V. A. Berezovskii, Hypoxia and Individual Differences in Reactivity [in Russian], Kiev (1978).
4. V. B. Zagustina, Z. A. Aleksanyan, and N. N. Vasilevskii, Usp. Fiziol. Nauk, **17**, No. 4, 68 (1986).
5. B. A. Karimov and N. I. Maisov, Pharmacological Correction of Hypoxic States [in Russian], Izhevsk (1988), pp. 55-56.
6. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System [in Russian], Moscow (1980).
7. G. N. Kryzhanovskii, A. A. Shandra, R. F. Makul'kin, and L. S. Godlevskii, Byull. Éksp. Biol. Med., No. 5, 527 (1985).
8. G. N. Kryzhanovskii, A. A. Shandra, R. F. Makul'kin, et al., Byull. Éksp. Biol. Med., No. 7, 18 (1989).
9. M. Ya. Maizelis, F. Z. Meerson, E. M. Leikina, et al., Byull. Éksp. Biol. Med., No. 1, 28 (1970).
10. A. A. Shandra, L. S. Godlevskii, and N. D. Semenyuk, Byull. Éksp. Biol. Med., No. 4, 20 (1983).
11. N. A. Agadzhanyan (N. A. Agadjanian), I. G. Vlasova, and V. I. Torshin, Acta Neurol., **7**, No. 3/4, 271 (1985).
12. R. G. Dieh, A. Smialowski, and T. Gotwo, Epilepsia, **25**, No. 4, 506 (1984).
13. D. S. Goldstein, N. S. Nadi, R. Stull, et al., J. Neurochem., **50**, No. 1, 225 (1988).
14. G. N. Kryzhanovskii (G. N. Kryzhanovsky), Central Nervous System Pathology: a New Approach, Mew York (1986).
15. G. N. Kryzhanovkii (G. N. Kryzhanovsky), A. A. Shandra, L. S. Godlevskii (L. S. Godlevsky), and M. Y. Karganov, Epilepsia, **30**, No. 5, 631 (1989).