Changes in the Level of Cyclic Nucleotides in the Left and Right Sensorimotor Regions of the Rat Cortex after Pentylenetetrazole Kindling

R. N. Glebov, M. N. Karpova, T. V. Goryacheva, I. O. Mikhal'skaya, M. E. Spasskaya, and O. Yu. Pankov

UDC 616.8-009.24-092-07:
616.831.31-008.939.633

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.116, № 11, pp. 491-493, November, 1993
Original article submitted June 17, 1993.

Key Words: *pentylenetetrazole kindling; rat cortex; cyclic nucleotides; right and left sensorimotor cortex*

It is known [5] that the seizure state (SS, kindling) is accompanied by plastic reorganizations in neurons, which preserve the "structural trace" for a long time. These reorganizations may be initiated by cyclic nucleotides, which provide for the posttranslation modification of functionally important proteins and enzymes due to phosphorylation of potential substrates.

Significant changes in the dynamics of development both of epileptic activity (EpA) and of SS occur in the cyclic nucleotide contents in certain structures of the brain, usually directed toward an increase of the cAMP and cGMP concentration (a variability is noted in the latter case, depending on the dynamics of the process and on the region of the brain) [1]. It is to be noted that data on kindling are few in number and contradictory.

In 1990 Japanese scientists shed new light on this problem; they showed that, whereas functional asymmetry in cAMP distribution is normally absent (in healthy rats), there is cortical asymmetry of norepinephrine-induced accumulation of cAMP (specified, however, by the correlation between the determinant and the "secondary" EpA in the right and left hemispheres [9]) in prolonged, 1-3 month EpA induced by $FeCl_2$ injected in the rat cortex.

The aim of the present investigation was to examine the cAMP and cGMP content in the sensorimotor region of the left and right hemispheres of the rat brain after the termination of chemical (pentylenetetrazole) kindling.

MATERIALS AND METHODS

Experiments were carried out on 100 male Wistar rats. Before the experiments the animals were tested according to a method we developed [4] in order to examine their sensitivity to pentylenetetrazole using a one-trial injection of the epileptogenic drug in the minimal effective dose of 40 mg/kg. According to their reaction, relatively homogeneous groups of sensitive animals (SA) and low-sensitive animals (LSA) were formed. The animals exhibiting a convulsive reaction were considered as being relatively more sensitive to the epileptogenic effect and they were used in further study of the effects of calcium antagonists on the formation of the SS. The animals without a convulsive reaction to the indicated dose were specified as low-sensitive. Kindling was performed one week after test-

Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0011-1368$12.50

TABLE 1. Content of cAMP and cGMP (pmole/g tissue) in Sensorimotor Cortex of Rat Brain 7 Days after Kindling

Group of animals and experimental conditions	cAMP		cGMP	
	L (a)	R (b)	R (c)	L (d)
1st – control, administration of 0.14 M NaCl for 30 days	0.23 (10)	0.28 (10)	0.36 (7)	0.33 (7)
2nd – control, one–trial administration of 0.14 M NaCl	0.12 (10)	0.13 (10)	0.11 (10)	0.13 (10)
3rd – one–trial injection of 60 mg/kg pentylenetetrazole	0.12 (6)	0.09 (6)	0.13 (6)	0.17 (6)
4th – kindling, SA	0.50 (4)	0.27 (4)	1.24 (4)	0.05 (4)
5th – kindling, LSA	0.47 (4)	0.21 (4)	0.10 (4)	0.38 (4)
6th – kindling + nifedipine, SA	0.21 (5)	0.23 (7)	0.37 (4)(4)	0.54
7th – kindling + IOS–1.1212, SA	0.20 (4)	0.15 (5)	0.45 (4)	0.33 (5)

Note. Animals were killed 7 days after the last injection of the drugs and 0.14 M NaCl. The number of experiments is in parentheses. R – right, L – left hemisphere. The results are significant for cAMP after the U test for the pairs: 1a–4a, 2a–4a, 1b–2b; for cGMP the pairs are: 4c–4d, 4d–6d, 1c–2c, 1d–2d. The values for the control with daily injections of dimethylsulfoxide were the same as for the 1st group.

ing by pentylenetetrazole injection every day i.p. in a subconvulsive dose of 30 mg/kg during 30 days. There were eight experimental series. In the first series pentylenetetrazole was injected in LSA (15 rats), and in the second in SA (15 rats). 1,4-Dihydropyridines were dissolved in dimethylsulfoxide and administered i.p. 30 min before every pentylenetetrazole injection as follows: nifedipine in a dose of 10 mg/kg (3rd series, 10 rats) and IOS-1.1212 in a dose of 2 mg/kg (4th series, 12 rats). Control animals were injected with dimethylsulfoxide in the same volume and under the same conditions (5th series, 10 rats) and with saline (6th series, 10 rats). A one-trial injection of saline was performed in the 7th series (10 rats) and of pentylenetetrazole in a convulsive dose of 70 mg/kg in the 8th series (10 rats). The severity of seizures in response to pentylenetetrazole injection was assessed every day using a six-point scale [4].

Animals were decapitated 7-10 days after the last injection or one-trial administration of pentylenetetrazole or saline, after which a portion of the sensorimotor cortex (15 mg) was isolated and put on ice, and then homogenized at 0-4°C on Tris-EDTA buffer, pH 7.4. The homogenate was boiled for 3 min and centrifuged after cooling (10 min, 5000 g), and the supernatant was freeze-dried and stored at -20°C for no more than 30 days. Assays of cAMP and cGMP were performed using Amersham kits (UK) with radioimmune techniques.

Data were processed statistically using the nonparametric *U* test (Mann-Whitney-Wilcoxon).

RESULTS

The level of cyclic nucleotides in the rat neocortex is unchanged 7 days after one-trial injection of pentylenetetrazole in comparison with the corresponding control (Table 1). This fact substantiated the choice of the period (7 days) after the end of kindling when there would be no changes of the studied biochemical parameters induced by the postconvulsive reaction to the last administration of pentylenetetrazole.

Control animals injected daily with saline during 30 days exhibit a two-threefold increase of the cAMP and cGMP levels in the neocortex in comparison with one-trial injected animals, which may be considered a result of so-called "injection stress." A rise of the level of cyclic nucleotides in different regions of the brain has also been noted by other authorities in different types of stress [6-8]. There is no asymmetry in the distribution of cyclic nucleotides in the sensorimotor region of the cortex in animals of either group. It should be noted that an asymmetry in the distribution of cAMP [9,11] and cGMP [11] in different regions of the brain, including the neocortex, is not normally observed in healthy rats, just as in our experiments.

After kindling, in the cortex of the SA compared to the animals with 30-day "injection stress"

the cGMP level rises in the right hemisphere and falls in the left; the cAMP level rises in the right hemisphere and does not change in the left hemisphere. Thus, there is a functional asymmetry in the distribution of cyclic nucleotides in the sensorimotor region of the rat cortex which is probably specific for kindling. There are no differences in brain cAMP level in the SA and LSA, but there is a tendency toward a difference in cGMP level (higher in the right and lower in the left hemisphere of the SA).

The administration of calcium antagonists during the formation of the SS did not cause any significant differences in the level of cyclic nucleotides in the brain, except for a change in the level of cGMP for nifedipine in the left hemisphere. The level of cyclic nucleotides in animals treated with calcium antagonists approximates that in animals with 30-day "injection stress." The insignificance of the differences may be related to the individual sensitivity of animals to calcium antagonists, which is reflected in the scatter of the obtained parameters. We showed previously that nifedipine in the dose used did not significantly affect the formation of the SS [2] and that IOS-1.1212 delayed the development of kindling for 1 day and attenuated the severity of convulsions from the 17th day [3]. The totality of the findings suggests that the Ca component is not the only limiting mechanism of the plastic neuronal changes taking place in kindling.

It has been shown that the electrostimulatory kindling of the amygdala in rabbits results in an elevation of the cAMP content in this structure and the hippocampus 2 weeks later [10]. The same period after electrostimulatory (medial septum) or chemical (administration of carbachole in the amygdala) kindling, an increase is noted of both cAMP and cGMP in different regions of the brain, including the neocortex [11]. The authors did not find asymmetry in the distribution of cAMP and cGMP in the brain of treated animals, but the changes of the level of cyclic nucleotides in the brain were more typical for EpA in kindling than for kindling itself. This disagreement with our findings may result from diferences in the mechanisms of SS formation: while electrostimulatory and carbachole kindling may form a SS due to initial hyperactivation (superexcitation) of neurons, pentylenetetrazole kindling may do the same by switching off the inhibitory effects.

Thus, the finding of a cortical asymmetry in the distribution of cyclic nucleotides poses a new problem of "epilepsy and the functional asymmetry of the brain."

This study was partially financed by the Russian fund for basic research (project code 93-04-7476).

REFERENCES

1. R. N. Glebov and G. N. Kryzhanovskii, *Usp. Fiziol. Nauk*, № 3, 83-107 (1984).
2. M. N. Karpova, O. Yu. Pankov, G. N. Kryzhanovskii, *et al.*, *Byull. Eksp. Biol. Med.*, **112**, № 9, 260-262 (1991).
3. M. N. Karpova, O. Yu. Pankov, R. N. Glebov, *et al.*, *Ibid.*, № 10, pp. 362-365.
4. G. N. Kryzhanovskii, M. N. Karpova, and O. Yu. Pankov, *Ibid.*, **110**, № 10, 348-350 (1990).
5. G. N. Kryzhanovskii, A. A. Shandra, L. S. Godlevskii, *et al.*, *Usp. Fiziol. Nauk*, № 4, 12-33 (1988).
6. R. L. De La Paz, S. R. Dickman, and B. I. Grosser, *Brain Res.*, **85**, 171-175 (1975).
7. V. Dinnendahl, *Ibid.*, **100**, pp. 716-719.
8. V. Dinnendahl and K. Stock, *Naunyn-Schmiedeberg. Arch. Pharmacol.*, **290**, 297-306 (1975).
9. Y. Hattori, A. Moriwaki, Y. Hayashi, *et al.*, *J. Neurochem.*, **54**, 518-525 (1990).
10. S. Nikkuni, Y. Numata, Y. Kaneko, *et al.*, *Neurochem. Res.*, **4**, 658-659 (1979).
11. C. G. Wasterlain, M. D. Fairchild, J. M. Bronstein, *et al.*, in: *Kindling* 3, New-York (1986), pp. 55-69.