PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Protective Effect of Adaptation to Hypoxia and Its Prolongation by Pharmacological Agents in Rats Prone to Audiogenic Epilepsy

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Cytophotometric determination of RNA in various brain structures of hypoxia-adapted and unadapted epilepsy-prone rats at different times after an epileptic seizure shows much smaller decreases in RNA levels and their much more rapid return to normal in the adapted than in the unadapted rats. The adaptation to hypoxia produces a marked anticonvulsive effect, and this effect is enhanced and prolonged considerably by pharmacotherapy.

Key Words: *adaptation to hypoxia; audiogenic epilepsy; therapy*

Adaptation to periodic hypobaric hypoxia has been shown to increase considerably the resistance of Krushinsky-Molodkina (KM) rats to audiogenic epilepsy [6-9], to which they are genetically predisposed. While it has been suggested that the activation of nucleic acid and protein syntheses and the elevation of RNA content in the brain that occur during adaptation may play a role in the antiepileptic effect of the latter [12], the question of how an audiogenic convulsive seizure might affect RNA concentration in neurons of various brain structures of hypoxia-adapted and unadapted animals has not been addressed. Nor is it known how long the anticonvulsive effect of adaptation is retained after a course of adaptation and whether this effect can be prolonged by pharmacological means.

Accordingly, the objectives of this study were to measure RNA levels in neurons of various brain nuclei at different times after an audiogenic epileptic seizure in hypoxia-adapted and unadapted animals, to see how rapidly the resistance to audiogenic epilepsy declines after the termination of adaptation, and to find out whether and to what extent the resistance can be prolonged by pharmacological agents.

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MATERIALS AND METHODS

The study was conducted on male KM rats weighing 160-180 g which all reacted to an audiogenic stimulus (an electric bell ring of 90 dB) by motor excitation that terminated in clonic convulsions. The ringing was stopped as soon as these were observed. Limiting the duration of acoustic stimulation prevents mortality and the development of extensive subdural hemorrhages in the rats [8,9], which makes the KM rat model a very convenient tool for evaluating the anticonvulsive effect of adaptation.

Adaptation of the test rats to periodic hypoxia was achieved by elevating them to an "altitude" of

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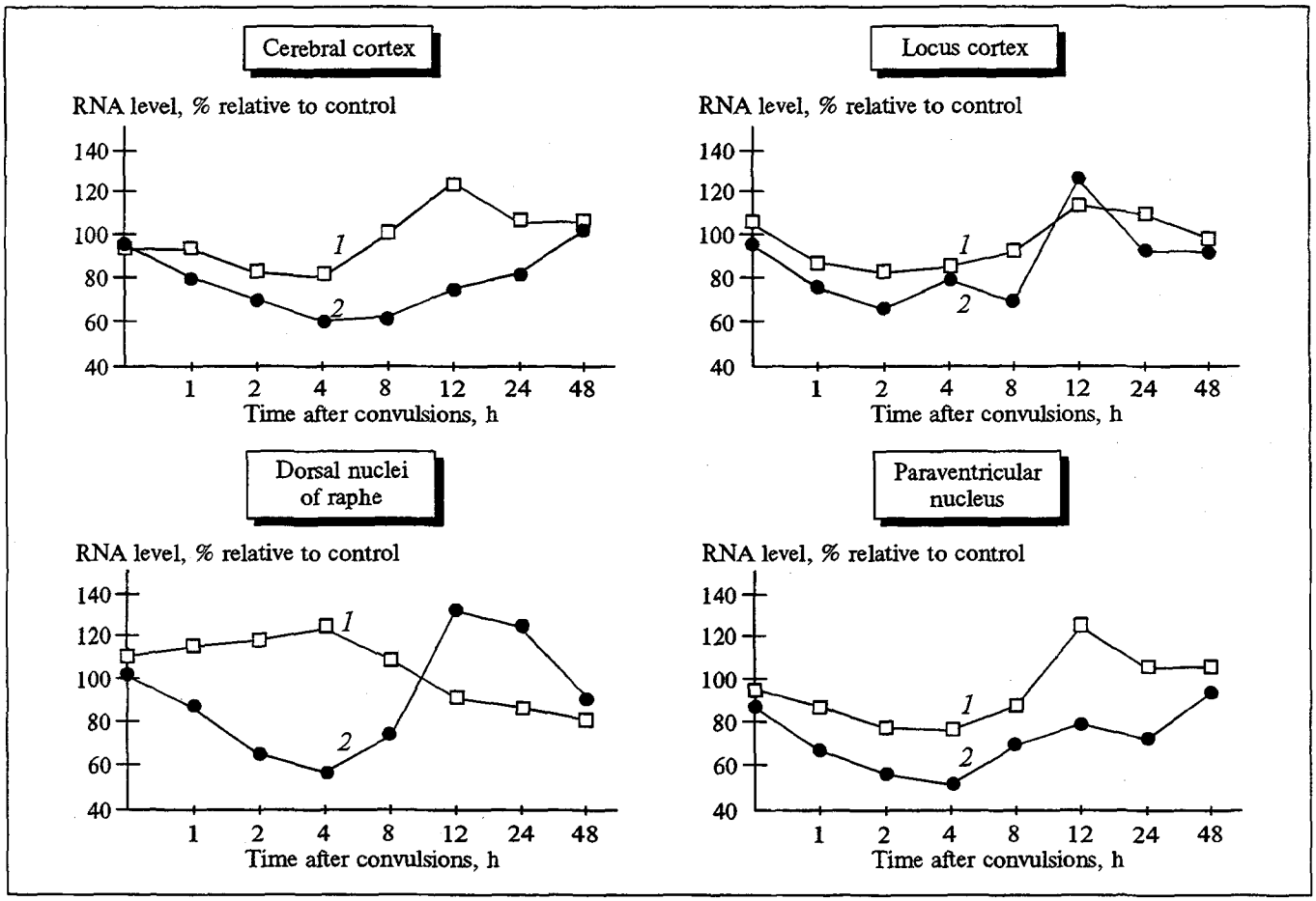

Fig. 1. Variation in cytoplasmic RNA levels in cellular brain structures after convulsions. *1*) hypoxia-adapted rats; *2*) unadapted rats.

5000 m in a pressure chamber, where they remained for 6 h per day for a total of 40 days. One day after the last elevation they were exposed to a bell ring and then decapitated either immediately or 1 to 48 h after the convulsions. The control group consisted of rats not adapted to hypoxia.

The brain structures selected for study were layer II of the temporal area of the cerebral cortex, locus ceruleus, dorsal nuclei of the raphe, and the paraventricular nucleus of the hypothalamus. Studies of the past two decades have proved that these structures play important roles in epileptogenesis [3].

The tissues to be tested were fixed in cold Carnoy fluid, subjected to the conventional histological treatment, and embedded in paraffin, after which serial 6-7 μ sections were prepared. An atlas of the rat brain was used to identify the nuclei [15]. The sections were stained for RNA with gallocyanin-chrome alum by Einarson's method as modified by Berub *et al.* [11]. The RNA concentration in the neuronal cytoplasm was determined with a cytophotometer at 525 nm by measuring absorbance of the dye bound stoichiometrically to the RNA. With this method, an accurate cytochemical analysis of cellular structures can be performed and quantitative metabolic changes can be detected in these structures. For each rat, 55 to 60 neurons were examined. The amount of RNA per cytoplasm of one neuron was expressed in percent relative to the intact control taken as 100% and calculated as the product of the volume of neuronal cytoplasm times its absorbance. The volumes of neuronal cytoplasm and nuclei were determined by formulas of triaxial ellipsoid and ellipsoid of revolution, respectively. This procedure has been described in detail previously [4]. All numerical results were treated statistically according to Student-Fisher.

For the cytochemical studies animals, which had responded to the ring of the bell with clonic convulsions, were used. Accordingly, the presence of such convulsions was the determining criterion of the protective-antiepileptic effect of adaptation.

To prolong the resistance to audiogenic epilepsy, three agents were used: Medopar-125, potassium orotate, and a combination of these two drugs. Madopar-125 (Farmakhim, Russia) was ad-

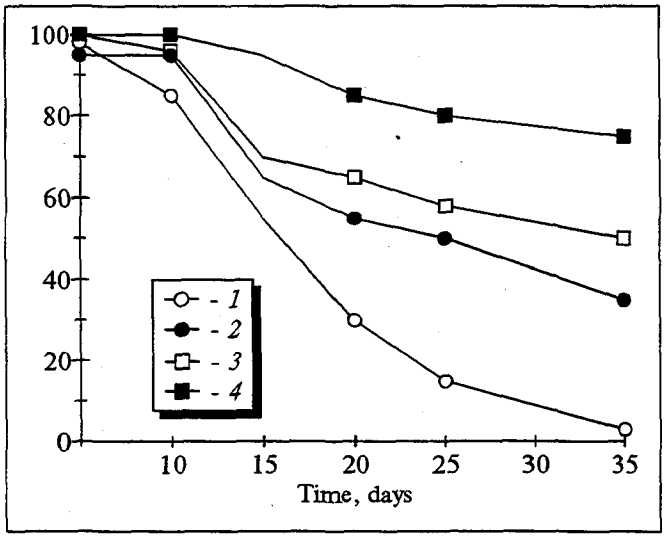

Fig. 2. Influence of activation of catecholaminergic systems on persistence of anticonvulsive effect of adaptation to hypoxia. Ordinate: percentage of rats that did not exhibit convulsions on days 5 to 35 after the last adaptation session. *1*) no drugs given; *2*) potassium orotate; *3*) Madopar – 125; *4*) Madopar – 125 + potassium orotate.

ministered intraperitoneally in a dose of 10 mg/kg in 0.14 mol/liter NaCl for 35 days after the termination of adaptation to hypoxia. Madopar-125 is a combination drug consisting of L-DOPA and benserazide. The latter prevents decarboxylation of L-DOPA in the blood and other tissues and promotes its entry into the brain [5]; benserazide itself does not cross the blood-brain barrier even in high concentrations. The use of L-DOPA in this way appeared justified because dopamine is synthesized from it and because the stress reaction [16] and in, particular, the severity of epileptic seizures [1,2] have been shown to be limited if the activity of the dopaminergic (stress-limiting) system in the brain remains high. Potassium orotate was administered orally in daily doses of 20 mg/kg for 35 days. This drug was chosen because orotic acid was expected to limit the reduction of the RNA content in the brain (such a reduction, as shown below, occurs under the influence of audiogenic seizures). In the combination Madopar-125 + potassium orotate, these two drugs were each used in the doses indicated above.

RESULTS

All control (unadapted) rats invariably responded to the bell ring by clonic convulsions. The adaptation had made the rats much more resistant to audiogenic stimulation: 65% of the test animals exhibited neither clonic convulsions nor circling movements, while 10% exhibited such movements only. In the remaining 25% of rats, circling was observed to give way to convulsive seizures, but these were of shorter duration than in the nonadapted rats; moreover, 10 to 15 min after the seizures the rats reacted appropriately to external stimuli, drank water, and did not differ from intact rats in motor activity. In the unadapted rats, the postictal period was marked by prolonged inhibition, absence of reactions to stimuli, and, in some cases, paresis of the hind limbs.

These observations agree well with those of other authors [8,9] who found that exposure of KM rats to hypoxia reduced considerably the postictal mortality and prevented motor disorders and brain hemorrhages, and that animals highly resistant to oxygen deficiency were similarly resistant to the convulsive action of penicillin and strychnine.

In the cytochemical study, we compared postictal RNA levels in the tested cellular structures of brains from unadapted rats and from those in which the adaptation had failed to prevent audiogenic convulsive seizures. The results are presented in Fig. 1. It can be seen that neuronal cytoplasmic RNA levels in the cerebral cortex (layer II of the temporal area) of unadapted rats were much lower at 2, 4, and 8 h after the audiogenic seizure than in the intact controls (by 31%, 39%, and 38%, respectively), and that they did not return to the control value until 24 h. In the adapted rats, the decrease in RNA at 2 h was only about half that in the nonadapted ones, and by 8 h the RNA level had reached the control value while at 12 h it even exceeded it by 20%. Similar effects of the adaptation on RNA occurred in the locus ceruleus, dorsal nuclei of the raphe, and paraventricular nucleus of the hypothalamus (Fig. 1). In these three brain structures, the adaptation limited or prevented the fall in RNA in the early postictal period and led to a rise of RNA to above-control values subsequently. The most striking result was obtained for the dorsal nuclei, where neurons of the GABA-ergic and serotoninergic systems are located. These systems (and their metabolites) have been shown to play important roles in limiting stress reactions and have therefore been designated "stress-limiting systems" [13].

It has been demonstrated that adaptation to hypoxia activates the GABA-ergic [10] and serotoninergic [14] systems so that the brain of adapted animals contains elevated levels of GABA and serotonin, whose anticonvulsive effects are well known [2].

In general, the data on RNA content in brain neurons of adapted and unadapted animals during the postictal period indicate that the epileptic

overexcitation of brain structures is consistently accompanied by falls in neuronal cytoplasmic RNA (which is mainly ribosomal RNA), and this may well be a factor limiting the synthesis of enzyme proteins and, in particular, of the major inhibitory neurotransmitters which are thought to play an important part in the pathogenesis of epileptic activity [3]. Adaptation to hypoxia prevents, largely or completely, the reduction in RNA and the associated disturbances in the protein-synthesizing systems. As a consequence, the activity of these systems increases, as do the levels of anticonvulsive metabolites of the stress-limiting systems, with the result that seizures either disappear or are curtailed.

The second stage of our work was designed to see how the resistance to audiogenic epilepsy declined after the last adaptation session in the pressure chamber and to what extent the anticonvulsive effect of adaptation to hypoxia could be prolonged by pharmacological agents. The results, shown in Fig. 2, indicate that the resistance waned rather rapidly. Thus, on day 15 after the last adaptation session, audiogenic convulsive seizures or circling movements in response to a bell ring were exhibited by 46% of the rats that had not developed them on day 1 after the last session; by day 35 the proportion of rats responding with convulsions or circling was as high as 95% (Fig. 2, curve *1*).

The administration of potassium orotate prolonged the anticonvulsive effect of adaptation: 40% of the orotate-treated rats did not develop convulsions vs 5% in the untreated group (Fig. 2, curve *2*). More effective in this respect was Madopar-125 (on day 35, no convulsions were observed in 50% of the rats - Fig. 2, curve *3*) and still more effective, the combination Madopar-125 + potassium orotate (on day 35 convulsions did not occur in 78% of the rats - Fig. 2, curve *4*).

In evaluating these results it is important to note that Madopar-125, potassium orotate, or their combination failed to protect by themselves: all rats that responded to acoustic stimulation by convulsions or circling continued to do so after treatment with either of these drugs or their combination. The only effect observed was a somewhat altered ratio of animals that responded to sound by convulsions (66%) to those that responded by circling (34%).

In other words, despite the agents, the liability to seizures constituted 100%. Thus, by using potassium orotate and Madopar-125, what we observed was not an independent antiepileptic effect of these agents, but rather an actual prolongation of the brain's resistance to the audiogenic epileptogen, due to the adaptation to hypoxia.

In conclusion, this study indicates that a combination of adaptation and pharmacotherapy is much more effective in preventing epileptic seizures than either of these approaches alone. This conclusion is in accord with the basic tenet of adaptive medicine that combining pharmacological and adaptive protection holds the greatest promise for this science.

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