CORRECTION OF OXIDATIVE METABOLISM OF CEREBRAL CORTICAL CELLS BY NICOTINAMIDE DURING KINDLING

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The writer showed previously that activity of certain NAD-dependent dehydrogenases in neurons and glial cells of the sensomotor cortex and hippocampus is depressed during the kindling phenomenon [2, 4]. Nicotinamide (NA) is an effective agent for stimulating oxidative metabolism. As a result of its use, the NAD concentration in various organs and tissues is restored to normal and dehydrogenase activity is increased, leading to stimulation of carbohydrate and lipid metabolism and to an increase in the animals' resistance to acute oxygen deprivation [8]. It has also been shown that NA is able to inhibit seizure activity [3].

Accordingly, in the investigation described below the possibility of using NA in doses giving a dual effect, namely correcting oxidative processes in brain cells and inhibiting seizure activity during kindling, was studied.

EXPERIMENTAL METHOD

Experiments were carried out on C57BLgo mice weighing 18-22 g. The animals were divided into four groups with at least 15-20 mice in each group. Kindling was induced with metrazol, which was injected intraperitoneally (30 mg/kg, once daily for 1 month). A full account of the method of producing kindling and of evaluating behavioral seizure responses was given previously [5]. Group 1 (control) consisted of mice receiving metrazol alone. Animals of the other groups also received metrazol by the schedule indicated above, but starting with the 4th week, they were given an intraperitoneal injection of NA solution in a dose of 5, 50, and 200 mg/kg for groups 2, 3, and 4 respectively, daily and 30 min before the injection of metrazol. At the end of the experiment the animals were decapitated, the brain was removed, and the regions for testing were taken from it. The control and experimental material was placed on the stage of a cryostat, frozen in liquid nitrogen, after which matched sections 10 μm thick were obtained in the cryostat chamber. Activity of glutamate- (GDH), succinate- (SDH), α-glycerophosphate- (GPDH), and glucose-6-phosphate- (G6PDH) dehydrogenases and also of NADH-tetrazolium reductase was determined histochemically by the method in [1]. Activity of these enzymes was determined in nerve and glial cells in five or six layers of the sensomotor cortex by single-wave cytophotometry at 585 nm. Morphometric investigations also were carried out on five groups of mice (the four mentioned above, and also group 5 consisting of intact animals). For this purpose the brain was fixed in formalin and embedded in paraffin wax. Sections 8 μm thick were stained with gallocyanin and chrome alum by Einarson's method [7]. The number of nerve cells was counted in layers 3-4 and 5-6 of the sensomotor cortex (in an area of 1023 μ^2 in each section) under a magnification of 40 $\times$ 10. Altogether 50 sections were studied from each group of mice.

EXPERIMENTAL RESULTS

The study of the action of NA on seizure responses of the mice showed that in doses of 50 and 200 mg/kg it depressed these responses by 10 and 13% respectively. A dose of 5 mg/kg of NA was ineffective (Table 1).

The results of the morphometric investigations showed a decrease in the number of neurons in the sensomotor cortex of the mice with kindling compared with the intact animals by 30% in layers 3-4 and by 19% in layers 5-6 (groups 1 and 5, Table 2). Comparison of the animals of group 1 with those receiving NA showed the following changes in the number of

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TABLE 1. Effect of Nicotinamide on Behavioral Seizures of Mice with Kindling ($M \pm m$)

Experimental conditions	Intensity of seizures, points
Control NA	3,0±0,08
5 mg/kg	3,04±0,09
50 mg/kg	2,69±0,07*
200 mg/kg	2,6±0,07**

Legend. $*p < 0.05$, $**p < 0.01$ compared with control.

TABLE 2. Changes in Number of Nerve Cells in Sensomotor Cortex of Mice with Kindling and Receiving Injection of NA ($M \pm m$)

Experimental conditions	Number of neurons per area of section	
	layers 3-4	layers 5-6
Control NA	30,33±0,61*	16,48±0,45*
5 mg/kg	31,12±0,49	16,69±0,32
50 mg/kg	33,7±0,79*	16,99±0,4
200 mg/kg	22,37±0,8*	12,78±0,36*
Intact mice	43,12±0,69	20,32±0,64

Legend. Data in group 1 are compared with those in group 5, data in groups 2, 3, and 4 with group 1. $*p < 0.001$ compared with control.

neurons in the latter: in mice of group 3 they were 11% more numerous in layers 3-4, whereas in mice of group 4, on the other hand, they were 26 and 22% less numerous respectively in layers 3-4 and 5-6.

The cytophotometric studies of enzyme activity in the brain cells of the mice of group 2, compared with the control, showed that GDH activity in the neurons was reduced by 8% ($p < 0.001$), and SDH and G6PDH activity by 7% ($p < 0.001$; Fig. 1), whereas GPDH activity in the neurons and glia was increased by 5% ($p < 0.05$) and 11% ($p < 0.001$) respectively; NADH-tetrazolium reductase activity in the same cells also was increased by 4% ($p < 0.05$) and 10% ($p < 0.001$) respectively. In the mice of experimental group 3 activity of the following enzymes was increased: GDH in the glia by 15% ($p < 0.001$), SDH in the neurons and glia by 9% ($p < 0.001$) and 29% ($p < 0.001$) respectively, and NADH-tetrazolium reductase in the same cells by 8% ($p < 0.001$) and 11% ($p < 0.001$) respectively. In addition to the changes mentioned above GPDH activity in the neurons and glia was reduced by 9% ($p < 0.01$) and 6% ($p < 0.01$). GDH activity in the neurons and glia of the mice of group 4 was reduced by 14% ($p < 0.001$) and 7% ($p < 0.001$) respectively, SDH activity in the same cells was reduced by 10% ($p < 0.001$) and 5% ($p < 0.05$), and G6PDH activity in the neurons also was reduced by 6% ($p < 0.05$). An increase in GPDH activity in the neurons and glia by 21% ($p < 0.001$) and NADH-tetrazolium reductase activity in the glial cells by 16% ($p < 0.001$) also was found.

The results of the experiments described above showed that in the experimental mice NA in a dose of 5 mg/kg induced dissimilar changes in enzyme activity in nerve and glial cells. The changes found are evidence that NA has a stimulating action of oxidative metabolism of the glia but no such action in neurons. Meanwhile this dose of NA was completely ineffective against seizure activity and did not protect nerve cells against the destructive action of the epileptogen.

When NA was given in a dose of 50 mg/kg changes in enzyme activity of the nerve and glial cells clearly reflected a general tendency toward stimulation of aerobic metabolism and inhibition of glycolysis. It must be emphasized that in the mice of this particular group changes in the glia were more significant than those in the neurons. Our data are in agreement with existing views on the stimulating action of NA in myocardial infarction [8] and of nicotinic acid in vascular pathology [6] on tissue oxidation. The morphometric parameters in the animals of this group (minimal loss of cortical cells) are evidence of increased viability of the neurons under the conditions of a seizure syndrome. It can be tentatively suggested that the

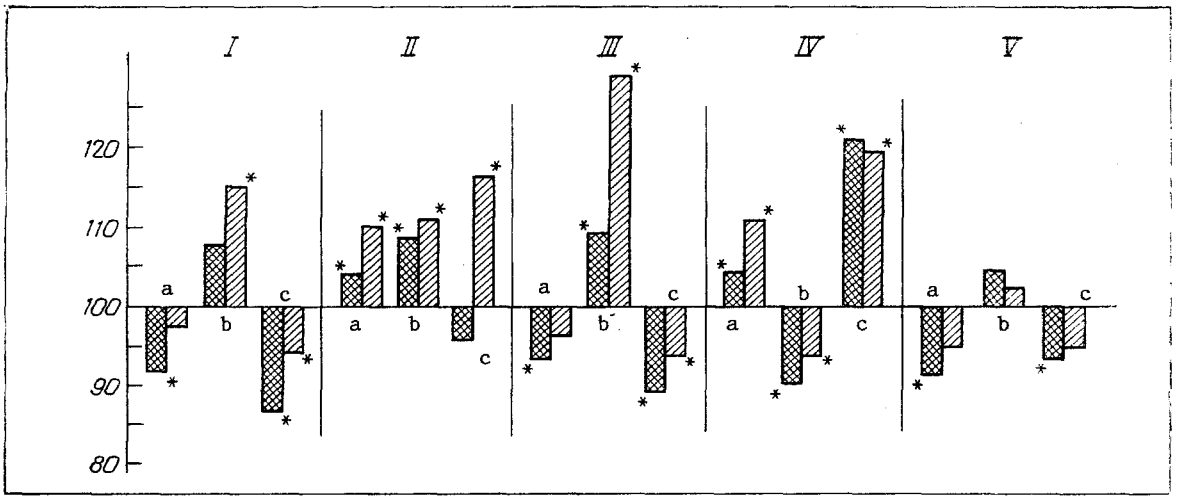

Fig. 1. Changes in enzyme activity in cerebral cortical cells of mice under the influence of NA during kindling. I, II, III, IV, V) GDH, NADH-tetrazolium reductase, SDH, GPDH, AND G6PDH respectively. Cross-hatched columns indicate neurons, obliquely shaded columns — glia. a, b, c) NA in dose of 5, 50, and 200 mg/kg respectively. Ordinate, enzyme activity (in % of control, taken as 100). Asterisk indicates that differences are significant.

regulatory action of NA on oxidative metabolism of neurons is one of the main mechanisms of the increased resistance of these cells to the destructive action of epileptogenic factors. The ability of NA to inhibit deacylation of phospholipids of membrane structures of brain cells, arising in stress situations, is evidently one of a number of protective mechanisms [9]. Finally, a positive factor in the action of NA was its definite anticonvulsant effect.

An increase in the dose of NA to 200 mg/kg had virtually no effect on the anticonvulsant action, but in this case changes in enzyme activity pointing to a negative effect of NA on neuronal and glial metabolism developed under these circumstances. These observations, and also the decrease in the number of neurons in the brain of the mice of this group, discovered morphometrically, suggest that in this dosage NA may have a toxic action on brain cells.

Comparison of the results of these investigations with those obtained by the writer previously leads to the conclusion that in mice with kindling there is a gradual and progressive increase in seizure activity, coupled with disturbances of oxidative metabolism [2], as a result of which destructive changes develop in the cerebral cortex and nerve cells die. The study of the therapeutic efficacy of NA in the seizure syndrome showed that an optimal dose of the compound can create a basis for realization of certain effects of its action on brain tissue: anticonvulsant, metabolic, and antidestructive. However, these actions are manifested only when high doses of the compound are given, substantially higher than the generally accepted values. Taking this into consideration, and also the possibility that high doses of NA may have a toxic action on brain tissue, their administration must be accompanied by careful monitoring of metabolism.

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