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EFFECT OF SOME VITAMIN PREPARATIONS ON EPILEPTIC ACTIVITY

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The development of rational treatment of epilepsy with the aid of natural metabolites is one of the most important trends in the problem of creating comprehensive pathogenetic therapy of this disease. A matter of great importance in such research is the choice of adequate models of epileptogenesis and, in particular, the production of states that closely resemble chronic epileptization of the brain. One such model is the method of pharmacologic kindling. In models of acute epileptic foci the writers previously demonstrated the effectiveness of nicotinamide, pyridoxal-5-phosphate, and α-tocopherol [3, 6]. The next important step was to study the effects of these substances on the development of epileptization of the brain on a kindling model.

The aim of this investigation was to study the effect of nicotinamide (NA), pyridoxal-5-phosphate (PP), and α-tocopherol (α-T) on developing epileptic activity (EA) and as a means of preventing its formation.

EXPERIMENTAL METHOD

Mice and rats were used. The animals received a daily intraperitoneal injection of a subconvulsant dose of metrazol (30 mg/kg). The EEG and behavioral responses were recorded. The intensity of seizures was expressed in points (for a detailed account of the technique, see [7]). NA and PP were injected in 0.1 ml of 0.9% NaCl solution 25 min, and an oily solution of α-T 24 h before injection of metrazol (in the group of animals with developed kindling). All drugs were injected intraperitoneally. Control animals in each series of experiments received an injection of physiological saline in the same volume as the test drug. The results were subjected to statistical analysis by variance and nonparametric methods.

EXPERIMENTAL RESULTS

During daily injections of metrazol progressive development of epileptization of the brain was observed, as shown by an increase in the number of animals with seizures and the greater severity of the seizure reactions from single shakings of the head and trunk to a

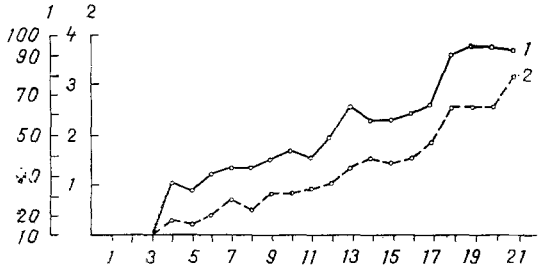

Fig. 1. Severity of seizure responses and number of animals with seizures evoked by subthreshold doses of metrazol. Abscissa, days of experiments; ordinate: 1) number of animals with seizures (in %); 2) severity of seizures (in points).

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TABLE 1. Effect of Nicotinamide (NA), Pyridoxal-5-Phosphate (PP), and α-Tocopherol (α-T) on Severity of Seizure Responses (M ± m)

Preparation administered	Dose, mg/kg	Ratio of number of animals with seizures to total number of animals		Severity of seizures, points	
		control	vitamins	control	vitamins
1. NA	100	30/30	20/31	3,2±0,2	2,7±0,2
2. NA	250	26/28	22/25	3,4±0,1	2,1±0,1*** $P_{1-2}<0,05$
3. NA	500	22/24	12/25	3,3±0,3	0,9±0,2*** $P_{3-2}<0,001$ $P_{3-1}<0,001$
4. NA	1000	19/20	7/25	3,1±0,3	0,4±0,1*** $P_{4-3}<0,05$
5. PP	10	14/16	10/12	2,9±0,4	2,8±0,5
6. PP	20	17/17	15/15	2,8±0,2	2,6±0,3
7. α-T	100	50/52	43/50	3,2±0,1	2,9±0,1*
8. NA + PP	250 10	28/31	16/43	2,9±0,2	1,6±0,2*** $P_{8-2}<0,05$
9. NA + PP + α-T	250 10	33/35	20/40	3,0±0,2	1,0±0,1*** $P_{9-8}<0,01$ $P_{9-2}<0,001$
10. NA + PP + α-T	500 10 100	17/20	8/20	2,9±0,3	0,4±0,03*** $P_{10-9}<0,001$ $P_{10-8}<0,001$ $P_{10-3}<0,02$

Legend. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control.

clonico-tonic convulsion, with the animal falling onto its side, secretion of saliva, defecation, and postictal depression (Fig. 1). A study of the EEG of the rats showed an increase in the intensity of EA in the cerebral cortex during development of kindling. The duration of the EEG changes also increased from 3-5 min on the first days of seizures to 1 h or more after 3 weeks of administration of metrazol.

The results of the study of the effect of a single injection of NA, PP, and α-T and of their combinations on seizures induced by injection of subconvulsant doses of metrazol in the course of 3 weeks are shown in Table 1. NA in doses of 250 and 500 mg/kg caused a decrease in the number of animals with seizures and a statistically significant decrease in the severity of the seizures. After injection of the larger dose of NA (1000 mg/kg) very weak seizures were observed in only one-third of the animals. PP (10 or 20 mg/kg) had no significant effect on the severity of the seizure responses or on the number of animals with seizures. Injection of α-T reduced the number of animals with seizures and the severity of the seizure respones. After injection of a combination of NA and PP and also of NA, PP, and α-T, a stronger anticonvulsant effect was observed than after administration of the preparations separately. The animals were divided into two groups after 3 weeks of daily injections of metrazol: group 1) daily injection of α-T (100 mg/kg), and injections of NA (250 mg/kg) and PP (10 mg/kg) 25 min before injection of metrazol; group 2 (control) — daily injections of physiological saline 25 min before metrazol. After 3 days of administration of the combination of vitamins, no seizures were present in half of the animals of group 1, and in the remainder the severity of the seizure responses was considerably reduced (Fig. 2). In the animals of group 2 the severity of the seizure responses at this time was 2.3 points (P < 0.01). On the 7th day of injection of the vitamins no seizures were observed in the animals of group 1, and on the 8th and 9th days only slight twitches were observed in two animals. To determine whether absence of seizures was the result of disappearance of the enhanced level of epileptization or simple suppression of manifestations of the seizure response under the influence of the combination of vitamins, animals of both groups were tested with metrazol after a pause of 10 days in administration of metrazol and the combination of vitamins. Although the mean severity of the seizures was a little greater in the animals of group 2 than in those of group 1, these differences were not statistically significant (P > 0.05; see Fig. 2, 20th day). During the next 10 days the animals of group 1 received daily injections of the combination of vitamins in the same doses. No metrazol was given to the animals of either group during this period. Subsequent injection of metrazol induced seizure responses in animals of both groups (see Fig. 2, 30th day). However, no seizures were present in some animals of group 1 (no vitamins were given on the day of injection of metrazol), and in the remaining animals of this group they are mild. The mean severity of the seizure response was less in group 1 than in group 2 (P < 0.05).

In the next series of experiments the possibility of preventing the development of epileptization by preliminary administration of NA, PP, and α-T was studied. Starting from

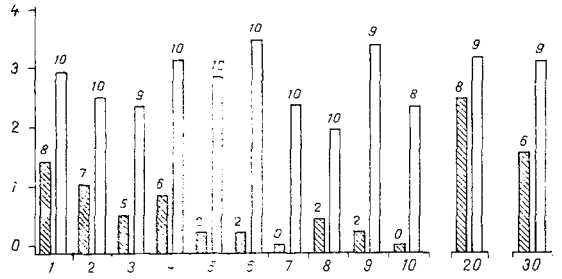

Fig. 2

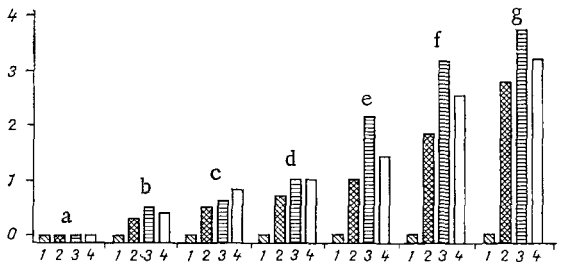

Fig. 3

Fig. 2. Effect of daily injection of vitamin preparations on severity of seizure response. Ordinate, severity of seizures (in points); abscissa, days of experiments. Unshaded columns — control; shaded — receiving vitamins. Numbers above columns indicate number of animals with seizures.

Fig. 3. Effect of NA, PP, and α-T on development of kindling. Abscissa, days of experiments; ordinate, severity of seizures (in points). 1) NA (250 mg/kg); 2) α-T (100 mg/kg); 3) PP (10 mg/kg); 4) control. a-g) 3rd, 6th, 9th, 12th, 15th, 18th, and 21st days respectively of experiments.

the first day of the experiments these vitamins were given before injection of metrazol. NA completely prevented the development of seizures in all animals (Fig. 3). α-T did not prevent seizures from developing and had no significant effect on the severity of the seizure response on nearly all days of observation. Only on the 19th and 20th days was the mean severity of the seizures in this group statistically significantly less ($P < 0.05$) than in the control. PP likewise did not prevent seizures from developing and had no significant effect on the severity of the seizures or the number of animals with seizures for 14 days. Starting with the 15th day, an increase in the severity of the seizure response was observed in this group ($P < 0.05$).

The results thus showed that during chronic administration of subconvulsant doses of metrazol the sensitivity of the brain to the epileptogen is enhanced and its epileptization increased. A single injection of subthreshold doses of metrazol into these animals induces generalized seizures. These results agree with those obtained by other workers [1, 14, 17], who showed that repeated injection of lidocaine or pentylenetetrazol into animals causes progressive development of seizures. It is important that this state of enhanced epileptization persists for a very long time, evidence of preservation of certain structural, physiological, and biochemical aftereffects that constitute the pathogenetic basis for maintenance of increased sensitivity of the brain to the action of the epileptogen. Despite this, a single dose of vitamin preparations was sufficient to depress the reactivity of the epileptized brain. Daily injections of a combination of vitamin preparations caused a more marked decrease in epileptization of the brain than a single dose of the vitamins. However, administration of vitamin preparations does not abolish epileptization which has already arisen, i.e., latent structural, physiological, and biochemical traces. To obtain a more lasting effect, further administration of vitamins is necessary. The results indicate the need for giving long-term vitamin therapy even in the absence of clinical manifestations of epileptic activity.

The fact that NA can prevent the development of kindling is very interesting. According to recent data, NA is a possible endogenous ligand of benzodiazepine receptors [15] and has an anticonvulsant action [3, 5]. It must be emphasized that under the experimental conditions described above nicotinamide was more effective than against acute epileptic foci or acute generalized metrazol convulsions [3, 6].

A special feature of the action of PP is that at certain stages of development of kindling it can intensify EA. It was shown previously that PP depresses EA of a certain nature [4]. It has also been shown that pyridoxine, a precursor of PP, raises the threshold of kindling induced by electrical stimulation of the amygdala [18]. Forms of pyridoxine-dependent epilepsy are known [10, 13]. The antiepileptic effect of PP, it can be tentatively suggested, is connected with increased GABA synthesis due to activation of glutamate decarboxylase. However, in experiments by the present writers [6] and others [12] it was shown that relatively high doses of PP may increase the severity of seizures. Under certain conditions

GABA and some of its analogs are known to be capable of potentiating EA [16]. It can be postulated that the proepileptic effects of PP are due to the fact that it is an indirect agonist of GABA, for it intensifies its synthesis. The proepileptic action of PP, moreover, was observed at stages when EA was already sufficiently well formed. Another possibility is that the formation of kindling is connected with the formation of endogenous epileptogens with high affinity for benzodiazepine receptors, which is enhanced by GABA [9]. Since PP can intensify GABA synthesis, this proepileptic effect of PP may be connected with increased activity of endogenous proepileptic ligands. It has also been shown that PP can increase GABA reuptake terminally, inhibit binding of GABA by receptors, and induce seizures [11].

When kindling had already developed, α-T caused a decrease in the intensity of the seizures, in agreement with results obtained previously [2, 6]. Considering that α-T, as an antioxidant [2], inhibits EA, there would seem to be a good case for including this preparation in the combined vitamin treatment of epilepsy.

All the facts and arguments described above support the previous conclusion [3, 6] that the use of a combination of vitamins (metabolites) is indicated for the treatment and prevention of epilepsy.

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