efficient of correlation between the features was 0.62 and the index of linearity of correlation between features (γ) was 0.028 ± 0.070.

The dynamics of the anticonvulsant action of phenazepam can thus be described by the following regression equation:

$$ED\ (\text{in } \mu g/kg) = (a + bt) \pm \Delta ED, \quad (2)$$

where $b = -1.143 \pm 0.316$ is the coefficient of regression (significant at the $P < 0.01$ level); $a = 374.22$ is the free term of regression; $\Delta ED = 32.6$ $\mu g/g$ is the error of representativeness of regression; and t the time of the experiment (in min).

Two other facts deserve attention in connection with the problem under discussion. First, the maximal anticonvulsant action of phenazepam was observed 10 min after its administration (Fig. 2) which did not coincide with the time of the maximum of the content of radioactive material in the brain (Fig. 1). The explanation is evidently the more complex interaction between these two parameters in the early period of investigation. Second, analysis of the radiochromatograms of chloroform extracts of the animals' brain showed (Fig. 3) that they contain two peaks of radioactivity which corresponded to the original preparation (I) and to its 3-hydroxy-derivative (II); their ratio in the brain was 3.5 : 1.

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FURTHER STUDY OF THE ANTIEPILEPTIC PROPERTIES OF NICOTINAMIDE

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KEY WORDS: nicotinamide; strychnine; penicillin; acetylcholine; epileptic activity; complex of epileptic foci; determinant structure.

Nicotinamide is a ligand for benzodiazepine receptors and has an action similar in some of its parameters to that of the benzodiazepines [12-14]. It has been shown that nicotinamide depressed epileptic activity both in a single focus and in a complex of epileptic foci induced in the cerebral cortex with strychnine [7].

The aim of this investigation was to study the antiepileptic action of nicotinamide on foci induced with the aid of substances disturbing different types of inhibition (strychnine, penicillin) or inducing direct depolarization of neurons (acetylcholine—ACH) [9-11, 16].

EXPERIMENTAL METHOD

Acute experiments were carried out on 24 cats. Under ether anesthesia the skin and subcutaneous cellular tissue were divided by a midline incision running from the nasal bones to the occiput. The eye was drained. By trephining the bones of the calvaria and orbit wide access was obtained to different parts of the frontal and temporo-parietal regions of the neocortex. The experiments began 1.5-2 h after administration of ether ceased. The animal was immobilized (tubocurarine 0.1 mg/kg) and artificially ventilated. Scattered

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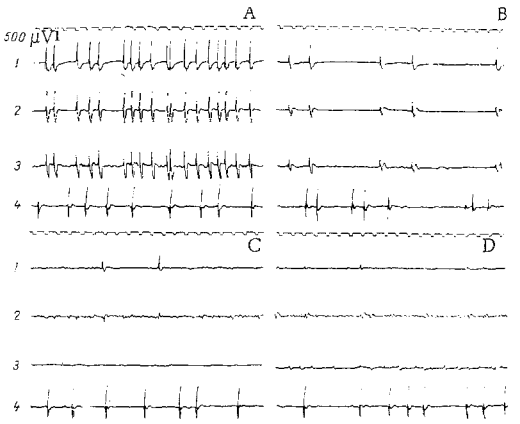

Fig. 1. Effect of nicotinamide on strychnine-induced epileptic complex and on isolated penicillin epileptic focus. A) Application of 0.1% strychnine to zone 2 and of 3% strychnine to zone 1 led to the creation of an epileptic complex: application of 1% penicillin to zone 4 created a focus generating discharges that were not synchronized with the activity of the complex; this focus remained isolated and independent. B, C, D) 2, 9, and 15 min, respectively, after intravenous injection of 300 mg/kg nicotinamide. 1) Anterior ectosylvian, 2) posterior sigmoid, 3) coronal, 4) middle lateral gyri. Signal calibration: 500 μV, time marker 1 sec.

foci of epileptic activity were created by application of a piece of filter paper (2 mm^2) soaked in 0.1% strychnine nitrate or in a 0.5-1% solution of the sodium salt of penicillin. Such foci were created in different parts of the anterior and posterior sigmoid and lateral gyri. A focus of powerful epileptic activity was formed in the orbital, coronal, and middle sigmoid or ectosylvian gyri by application of 3% strychnine solution or a 5-10% solution of ACH after preliminary treatment of this area of the cortex with 0.5% neostigmine. Brain potentials were derived by a monopolar method: The reference electrode was secured in the nasal bones and the active electrodes were cotton threads, soaked in Ringer's solution. Potentials were recorded on a 4-ÉÉG-3 ink-writing electroencephalograph. Nicotinamide was injected intravenously in a dose of 100-500 mg/kg.

EXPERIMENTAL RESULTS AND DISCUSSION

In the experiments of series I the effect of nicotinamide was studied on activity of a complex of epileptic foci created by application of strychnine solutions of different concentrations, and of a single penicillin focus. A few seconds after application of 0.1% strychnine solution to the cortex of the posterior sigmoid (zone 2) and coronal (zone 3) gyri and of 1% penicillin solution to the region of the middle lateral gyrus (zone 4) characteristic spike potentials appeared. After the appearance of paroxysmal discharges the pieces of filter paper with strychnine and penicillin were removed. The more powerful hyperactive focus created under these conditions in the anterior ectosylvian gyrus (zone 1) by application of 3% strychnine solution potentiated and synchronized activity in the foci of zones 2 and 3, united them into a single complex, and thus acquired the role of a determinant focus [1, 2]. After the appearance of a stable synchronized pattern of paroxysmal activity in the foci of the complex (Fig. 1A) the strychnine was removed from the region of the determinant focus. The penicillin focus did not join in the complex but continued to discharge independently according to its own pattern (Fig. 1A). As the control experiments and previous investigations [1-5] showed, an epileptic complex formed under these conditions can continue for 30-50 min, after which the amplitude and frequency of discharges in the foci decreased and the complex broke up. The single penicillin focus generated paroxysmal discharges for 1-2 h. Nicotinamide was injected at the stage of the most stable paroxysmal activity in all foci.

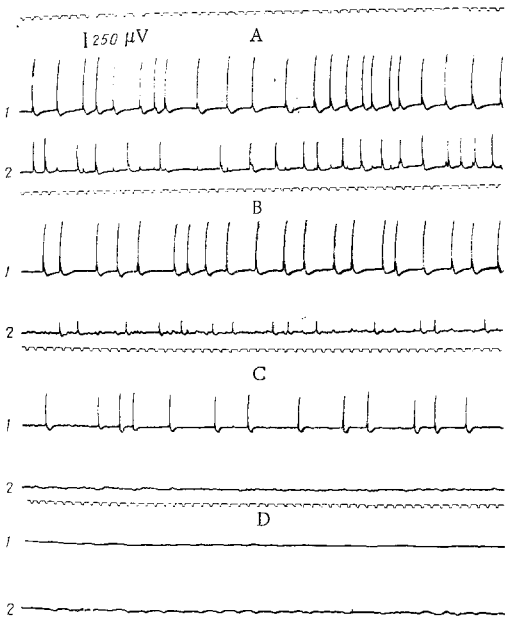

Fig. 2. Effect of nicotinamide on strychnine and penicillin foci. A) Application of 0.1% strychnine to zone 1 and of 0.5% penicillin to zone 2 led to the creation of epileptic foci; the pieces of filter paper with the convulsive agents were removed. B, C, D) 8, 12, and 18 min, respectively, after intravenous injection of 300 mg/kg nicotinamide. 1) Control, 2) anterior sigmoid gyri. Signal calibration: 250 μV, time marker 1 sec.

A marked decrease in amplitude and frequency of the discharges in all foci of the complex was observed 2-3 min after injection of nicotinamide; the greatest degree of inhibition of epileptic activity occurred initially in the focus farthest from the determinant focus (zone 3; Fig. 1B). Activity of the penicillin focus in this and subsequent periods did not change significantly. Epileptic activity in the dependent foci of the complex (zones 2 and 3) completely disappeared 9-10 min after injection of nicotinamide and it was considerably reduced in the determinant focus (zone 1; Fig. 1C), but after 15 min it disappeared in the determinant focus also (Fig. 1D), and remained only in the penicillin focus (until 2 h after injection of nicotinamide). In cases when the penicillin focus was created by brief (a few seconds) application of 0.5% penicillin, the spike potentials arising under these circumstances did not exceed 300-600 μV and were much smaller in amplitude than the discharges of the strychnine focus (Fig. 2A); after injection of nicotinamide a penicillin focus of this type was inhibited first of all and, moreover, completely (Fig. 2B, C).

In the next series of experiments the effect of nicotinamide was studied on activity of an epileptic focus created by means of ACH. A few minutes after application of 0.5% neostigmine and 5% ACH to the lateral gyrus characteristic paroxysmal discharges in the form of a high-amplitude positive–negative spike and a rhythmic after-discharge appeared (Fig. 3A). The piece of filter paper with ACH was removed after the formation of such a focus. Control experiments showed that the epileptic focus thus created generated paroxysmal discharges for a period of 40-60 min. Injection of nicotinamide, given immediately after removal of the filter paper with ACH, led to a considerable reduction in amplitude and, in particular, duration of the after-discharge after 2-6 min (Fig. 3B). During the next 7-12 min after injection of nicotinamide there was a marked decrease in amplitude of the spike potential and complete disappearance of the after-discharge (Fig. 3C), but after 18-20 min the paroxysmal discharges disappeared completely (Fig. 3D).

The effect of nicotinamide on generalized epileptic activity evoked by application of concentrated solutions of strychnine and penicillin, and characterized by the appearance of epileptic discharges in areas of the neocortex not treated with the convulsive substances, was studied in a separate series of experiments. In none of the experiments did injection of nicotinamide in a dose of 500-1000 mg/kg lead to depression of generalized epileptic activity, not did it prevent the onset of generalized seizures.

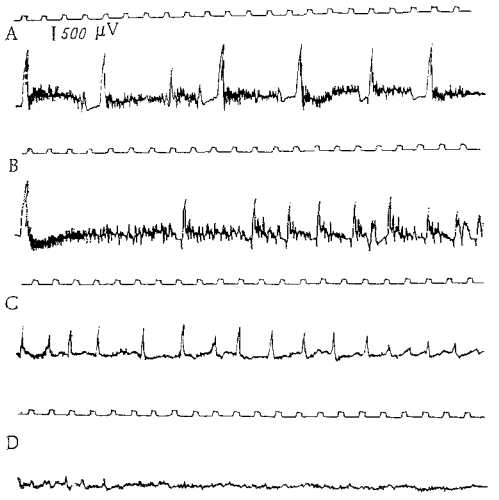

Fig. 3. Changes in activity of ACH focus under the influence of nicotinamide. A) Epileptic activity in focus created by application of 0.5% neostigmine and 5% ACH to lateral gyrus; filter paper with ACH removed. B, C, D) 2, 6, and 18 min, respectively, after intravenous injection of 500 mg/kg nicotinamide. Signal calibration: 500 μV, time marker 1 sec.

The intensity of the antiepileptic effects of nicotinamide thus depends largely on which inhibitory systems in the neuron population constituting the epileptic focus are blocked or weakened, and to what degree. We know that strychnine disturbs inhibition by blocking glycine receptors on the postsynaptic membrane [11]. Penicillin blocks GABA receptors on the post- and presynaptic membranes [10]. Meanwhile there is evidence that strychnine, penicillin, and other convulsants are not true competitors of glycine and GABA, and that the disturbance of inhibition is evidently due to blocking of membrane channels for chlorine ions [15]. Hyperactivity of epileptic neurons induced by ACH is connected with its direct depolarizing action [9].

On the basis of the foregoing facts it can be postulated that the antiepileptic effects of nicotinamide are realized through activation of the GABA-ergic apparatus and through GABA-inhibitory control. The experiments showed that nicotinamide primarily inhibits activity of a single focus and of a complex of foci created with the aid of strychnine, but has no marked effect on generalized strychnine activity. Lapin [8] likewise found no effect of nicotinamide on generalized metrazol and audiogenic convulsions.

Möhler et al. [12] showed that preliminary injection of nicotinamide (500 mg/kg) prevented the development of convulsions in 25% of animals induced by 3-mercaptopropionic acid, an inhibitor of GABA synthesis. The results of the present experiments are evidence that penicillin, by blocking GABA receptors, prevents the anticonvulsant action of nicotinamide. Meanwhile nicotinamide depresses epileptic activity in weak penicillin foci, where inhibitory mechanisms of GABA control are probably still preserved. It is also possible that the anticonvulsant effects of nicotinamide are connected not only with the inhibitory action of GABA. The intensity of the epileptic activity is of great importance: The higher its intensity the more difficult it is to inhibit the epileptic focus; this rule, moreover, is manifested in the case of both penicillin and strychnine foci.

It is an interesting fact that nicotinamide primarily depressed the rhythmic after-discharge and later the spike potential of ACH discharges. According to Ferguson and Jasper [15], the spike-wave and after-effect are generated by neurons in different layers of the cortex. The sensitivity of the latter to the action of nicotinamide thus differs. It can be tentatively suggested that this is connected with differences in the density of distribution of benzodiazepine receptors, with which nicotinamide binds specifically, in the cortical layers. The facts described above also point to the important role of choice of model with which to investigate the antiepileptic properties of nicotinamide.

On the whole, these investigations confirm the earlier hypothesis that nicotinamide is one of a number of endogenous antiepileptic agents which play a role in the regulation of epileptogenesis in the brain and which are capable of maintaining the activity of the antiepileptic system [7].

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ROLE OF THE GABA-ERGIC COMPONENT IN THE DEVELOPMENT OF THE TRANQUILIZING EFFECT IN CATS

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KEY WORDS: GABA-ergic component; antiphobic effect; n-dipropyl acetate.

GABA (if administered in a manner bypassing the blood–brain barrier) and its derivatives (the GABA-mimetic muscimol, inhibitors of GABA transaminase) [10] are known to exhibit an inhibitory effect on locomotor activity, investigative behavior, and conditioned reflexes in animals. Some structural analogs of GABA have found clinical application as tranquilizers (fenibut, sodium hydroxybutyrate, fepyron) [8]. The mechanism of action of the benzodiazepine tranquilizers is linked with their allosteric effect through specific receptors on the system regulating affinity of the GABA receptor for endogenous GABA [9]. However, the question of the role of the GABA-ergic component in the development of the tranquilizing effect is not yet clear. On the psychophysiological plane the tranquilizing effect is manifested as a broad spectrum of behavioral changes in which emotional, activating, and sedative components can be distinguished; consequently, to assess the role of the GABA-ergic component in the manifestation of the tranquilizing effect on behavior, models taking into account the complex structure of this phenomenon must be used.

The aim of the present investigation was to evaluate changes in the spectrum of emotional-behavioral reactivity of animals (cats) under the influence of n-dipropyl acetate (n-DPA), which raises the brain GABA concentration [12], by comparison with diazepam, which has a similar effect on the brain GABA concentration [6].

EXPERIMENTAL METHOD

The spectrum of emotional-behavioral reactivity was assessed in chronic experiments on cats by the method described in detail previously [3]. A state of anxiety and fear was induced by stimulation of the emotiogenic zones of the hypothalamus through implanted electrodes, by peripheral electrical stimulation of the skin, or by placing the animal a second time in an emotionally meaningful aversive situation (conditioned-reflex fear response) [5]. Interpretation of the significance of the response manifestations and their quanti-

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