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EFFECT OF NICOTINAMIDE ON EPILEPTIC ACTIVITY IN THE CEREBRAL CORTEX

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It has been shown [14] that nicotinamide is an endogenous agent or ligand which can bind specifically with benzodiazepine receptors found in the brain [13, 17] and give rise to a benzodiazepine-like effect, as reflected in various indices and, in particular, it can influence certain forms of activity of the spinal reflex apparatus [14]. Nicotinamide is known to prevent reactions catalyzed by glutamate decarboxylase, i.e., to cause insufficiency of GABA synthesis [14].

In the investigation described below it was shown that nicotinamide can suppress electrical activity both in the single focus and in complexes of epileptic foci evoked in the cerebral cortex. The use of an epileptic complex to study the effect of nicotinamide is of special interest, for the complex is a model of a pathological epileptic system arising from foci with comparatively low initial activity under the influence of a powerful determinant focus, which intensifies excitation in other foci, synchronizing their activity, and unites them into a single complex, determining the character of its activity [3, 4]. In such a system, its separate parts (foci) are functionally unequal and they behave differently toward inhibitory procedures [4-6].

EXPERIMENTAL METHOD

Acute experiments were carried out on 12 cats. Under ether anesthesia the skin and subcutaneous cellular tissues were divided by a midline incision running from the nasal bones to the occiput. The eyeball was drained. The cranial bones and orbit were trephined to give wide access to different parts of the frontal and temporal cortex, after which administration of ether was discontinued. The animal was immobilized (with 0.5-1 mg/kg diplacin) and artifically ventilated. Scattered foci of epileptic activity were created by application of filter paper (2 mm^2) soaked in 0.1-0.5% strychnine solution. These foci were formed in different parts of the coronary, anterior and posterior sigmoid, and ectosylvian gyri. A focus of powerful epileptic activity was created in the orbital or coronary gyri by application of a 1-3% solution or a crystal of strychnine. Biopotentials were derived by a monopolar electrode, the reference electrode was fixed in the nasal bones, and cotton threads soaked in Ringer's solution were used as active electrodes. Potentials were recorded on a 4-EEG-3 ink-writing electroencephalograph. A 5% solution of nicotinamide was injected intravenously in a dose of 50-100 mg/kg, but in some experiments larger doses (500-800 mg/kg) were used.

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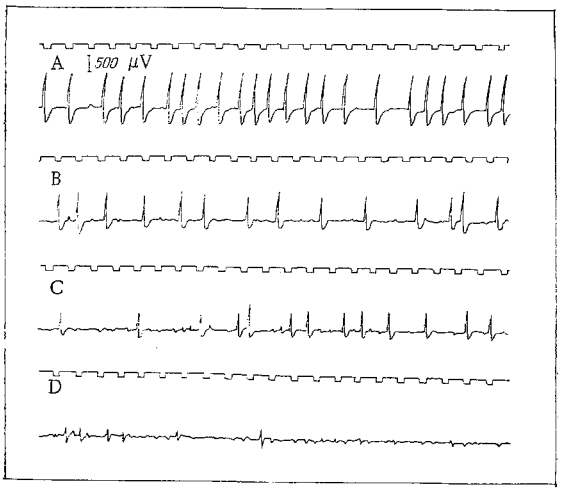

Fig. 1. Effect of nicotinamide on a single focus of epileptic activity. A) epileptic activity in focus created by application of 1% strychnine solution to posterior sigmoid gyrus; 4 min after application, strychninization discontinued (filter paper with strychnine removed); B) 10 min, C) 15 min, D) 18 min after intravenous injection of 50 mg/kg nicotinamide. Calibration signal 500 μV, time marker 1 sec.

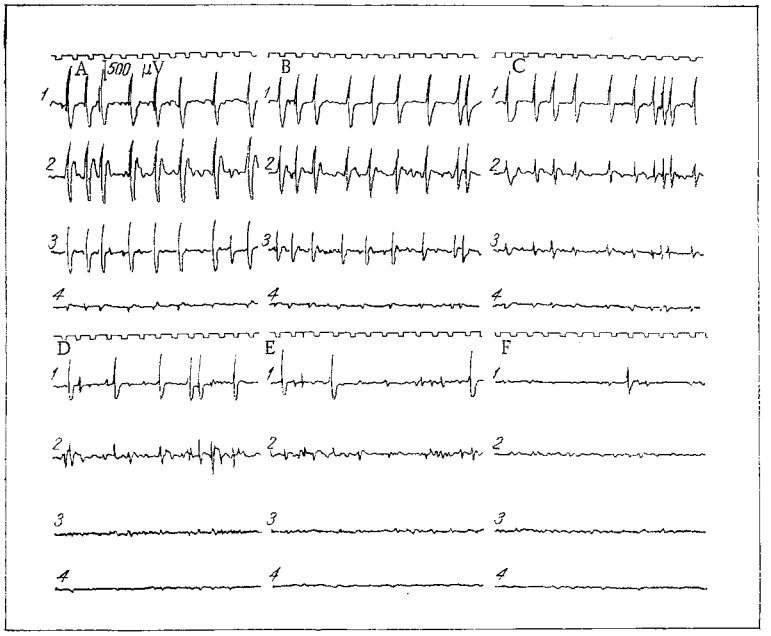

Fig. 2. Effect of nicotinamide on epileptic complex. A) epileptic complex created by application of 0.1% strychnine solution to zones 2 and 3 and application of 3% strychnine solution to zone 1 (determinant focus); 3 min after stopping application of strychnine to region of determinant focus; B) 2 min, C) 4 min, D) 7 min, E) 13 min, and F) 28 min after intravenous injection of 50 mg/kg nicotinamide. 1) coronary gyrus, 2) posterior and 3) anterior sigmoid gyri, 4) ectosylvian gyrus. Calibration signal 500 μV, time marker 1 sec.

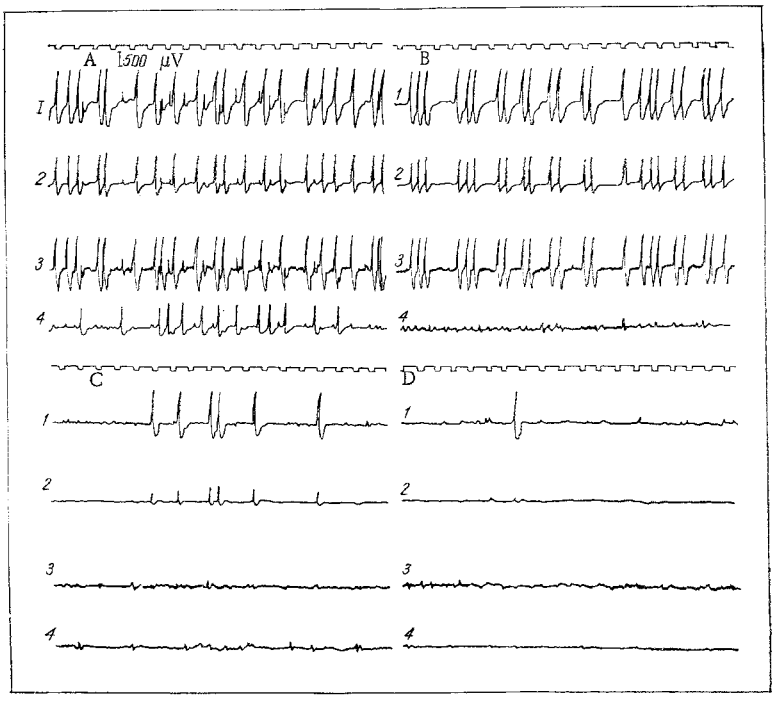

Fig. 3. Effect of nicotinamide on epileptic complex and on isolated epileptic focus. A) 14 min after application of 0.5% solution to zones 2, 3, and 4 and of 3% solution of strychnine to zone 1. Epileptic complex thus created consists of foci in zones 1, 2, and 3. Paroxysmal discharges in these foci are of high amplitude and hypersynchronized. Discharges in focus 4 are of lower amplitude and not synchronized with activity of the complex; this focus remains isolated and independent; B) 10 min, C) 16 min, and D) 25 min after intravenous injection of 80 mg/kg nicotinamide. 1) orbital, 2) coronary, 3) posterior, and 4) anterior sigmoid gyri. Calibration signal 500 μV, time marker 1 sec.

EXPERIMENTAL RESULTS

In experiments to study the effect of nicotinamide on a single epileptic focus, the latter was created by application of 1% strychnine solution to the posterior sigmoid gyrus. Characteristic strychnine potentials appeared at the site of application of strychnine a few seconds after its application. The piece of filter paper with strychnine was removed 4-5 min after application. Control experiments showed that the focus of paroxysmal activity thus created continued to generate spike discharges for 30-50 min. Intravenous injection of nicotinamide solution, given immediately after removal of the piece of paper with strychnine, caused a sharp decrease in the amplitude and frequency of the paroxysmal discharges in the focus after 5-10 min (Fig. 1B). During the next 12-18 min there was a further decline in the paroxysmal discharges, followed by their disappearance (Fig. 1C, D).

In the experiments to study the effect of nicotinamide on the epileptic complex, the latter was created by the accepted method described previously [3], namely by application of strychnine solutions of different concentrations to different areas of the cortex: The posterior (zone 2) and anterior (zone 3) sigmoid gyri were treated with 0.1% strychnine solution. After the appearance of paroxysmal discharges the pieces of filter paper with strychnine were removed. A more powerful hyperactive focus was then created in the coronary gyrus by application of 3% strychnine solution. This focus acquired a determinant role [3]: It potentiated and synchronized activity in the other foci and united them into a complex, which functioned as a single unit. After establishment of a steady state of synchronized paroxysmal activity in all the foci of the complex (Fig. 2A) the strychnine was removed from the region of the determinant focus. Control experiments, together with numerous previous investigations [3-6], showed that the epileptic complex formed under these conditions, consisting of three foci, could generate synchronous paroxysmal discharges for a period of 30-50 min, after which the amplitude and frequency of the discharges in the foci were reduced and the complex disintegrated. Injection of nicotinamide

at the stage of maximally stable, synchronous paroxysmal activity of the complex led to suppression of the paroxysmal discharges. A reduction in amplitude of the discharges was observed as early as 3-5 min after injection of the nicotinamide to begin with and more especially in the focus located farthest from the determinant focus (zone 3) and also in the other dependent focus (zone 2). In the determinant focus, however, at this period there were no significant changes in the character of the paroxysmal activity (Fig. 2B). Later, 7-15 min after injection of nicotinamide, the paroxysmal discharges disappeared in zone 3 and were considerably reduced in zone 2 (Fig. 2C, D), and after 13-25 min they disappeared in all the dependent foci (Fig. 2E), and later in the determinant foci also (Fig. 2F).

In cases when one of the foci did not join at the complex but continued to operate independently, it was the first to be suppressed after injection of nicotinamide, and its suppression was complete (Fig. 3B, zone 4). This phenomenon of weak resistance of the single focus compared with the epileptic complex also was demonstrated during testing of the antiepileptic action of diazepam. Later the reduction and disintegration of the complex under the influence of nicotinamide took place in the order described above (Fig. 3C); the determinant focus, which generated single paroxysmal discharges (Fig. 3D), was most resistant.

The effect of suppression of the complex of epileptic activity by nicotinamide depended on the dose of the compound: The higher the dose the more rapidly complete suppression of the complex, including the determinant focus, took place. When large doses (500-800 mg/kg) were used, a complete inhibitory effect appeared within a few minutes. In some experiments suppression of paroxysmal activity of the determinant focus was incomplete, and 30-40 min after injection of the compound (50 mg/kg) an increase in the amplitude and frequency of the paroxysmal discharges could take place, and they continued to be generated for a further 20-30 min. This result is also evidence that disappearance of the epileptic activity in the foci was due to the action of the nicotinamide and not to natural cessation of the strychnine effect.

In separate experiments in which a determinant focus was created by application of strychnine crystals, and dependent foci were created by application of 0.5% strychnine solution, injection of nicotinamide in a dose of 50-70 mg/kg was accompanied only by a decrease in the amplitude and frequency of the paroxysmal discharges and by disturbance of their synchronization. The complex as such disintegrated and in its place the separate foci, working independently of each other on autonomous regimes, remained. However, activity of the foci was not completely suppressed, and 30-40 min after injection of the nicotinamide an increase in amplitude of the discharges was observed in the determinant and dependent foci with restoration of activity of the complex.

The results of these investigations thus show that nicotinamide, when injected intravenously, depresses epileptic activity both in a single focus and in foci combined into an epileptic complex under the influence of a determinant focus. In all cases the same sequence of disappearance of epileptic activity was observed: To begin with it disappeared in the dependent foci, and it did so sooner in the focus farthest removed from the determinant focus, and in which the activity was relatively weaker; later activity disappeared in another focus lying nearer to the determinant focus. Last of all, epileptic activity was suppressed in the determinant focus, and larger doses of nicotinamide were required to suppress it completely. Together with these changes, destabilization of the whole complex took place in the foci and it disintegrated as a pathological system.

The order of suppression of the foci of the complex and the special features of its reduction and disintegration observed in these experiments were not a specific result of the action of nicotinamide. A similar effect arises through the action of diazepam and of general anesthetics [4, 5], and it is brought about by cortical mechanisms proper, for it is also observed in preparations of the isolated cortex [6]. This rule governing suppression of the epileptic complex is thus universal and is determined by the character of functional organization of the epileptic complex as a system.

Since nicotinamide binds specifically with benzodiazepine receptors [13, 17] and since, like diazepam, it can suppress epileptic activity and also give rise to other effects, it can be tentatively suggested that the mechanisms of its action are similar to those of diazepam: They are realized through activation of the GABA-ergic apparatus and to inhibitory control by GABA.

It can thus be suggested that nicotinamide plays the role of an endogenous substance contained in the brain – a ligand evoking a diazepam-like effect. The fact that in these experiments nicotinamide suppressed epileptic activity when administered systemically in comparatively high doses (in particular, in much higher doses than diazepam), may be explained by many factors. It is evidently connected with the rapid metabolism and poor penetration of nicotinamide into the brain, a characteristic feature of natural biologically active substances playing the role of neuromediators or neuroregulators. It has been shown that only a 0.3% solution of nicotinamide can penetrate into the brain when injected by the intracarotid route [9].

It is a very interesting fact that nicotinamide [8, 16], like benzodiazepine in small doses [15, 18], can potentiate certain components of paradoxical sleep which, as we know, suppresses epileptic activity [7, 10, 19].

On the basis of the above remarks it can be postulated that nicotinamide is an endogenous antiepileptic agent which plays a role in the regulation of electrogenesis in the brain. It can be regarded as the chemical equivalent of activity of the antiepileptic system, which is evidently capable of maintaining that activity. At the same time, its action cannot be limited to the suppression of foci in epilepsy: It may also be effective in other neuropathological syndromes characterized by hyperactivity of systems as a result of disturbance of inhibitory mechanisms and the creation of generators of pathologically enhanced excitation [1, 2].

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