EFFECT OF NICOTINAMIDE ON FOCAL AND GENERALIZED CORTICAL EPILEPTIC ACTIVITY

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Evidence has been obtained to show that nicotinamide is one possible endogenous ligand for benzodiazepine receptors and that its action, in a number of respects, is similar to that of diazepam [11-14]. Nicotinamide has been shown to depress both single epileptic foci and their complexes in the cerebral cortex [5, 6]. Meanwhile, in some cases nicotinamide had no marked effect on epileptic activity (EA) [6].

The object of the present investigation was to discover whether nicotinamide affects focal and primary generalized EA.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 180-220 g were used. On the day before the experiment, under hexobarbital anesthesia, the skull was exposed, two burr-holes were drilled above symmetrical areas of the sensomotor cortex of both hemispheres, and pieces of dura the same size as the burr-holes were removed. To record the electrocorticogram (ECoG) silver ball electrodes were fixed 0.5 mm in front of the burr-holes so that they were in contact with the intact part of the dura near the anterior edge of the exposed part of the cortex. The reference electrode was secured in the nasal bones. "Noracryl" paste was applied around the burr-holes to form a capsule after hardening. The capsule was filled with 0.85% NaCl solution and a waterproof film made an airtight cover over it. On the day after the operation, the animals were semirestrained in a frame which did not restrict movements of the head and limbs and was nontraumatic. A focus of EA was created in the cortex by application of a solution of the sodium salt of penicillin to the exposed cortical surface. In the experiments of series I, penicillin was applied simultaneously to both symmetrical areas of the sensomotor cortex, and in this case electrical activity of the two foci was completely synchronized. In the experiments of series II, penicillin was applied to one hemisphere and physiological saline to the symmetrical area of the opposite hemisphere. In this case, a focus of EA appeared directly in the zone of application of penicillin (the determinant focus [3]), and a dependent, or mirror focus of EA appeared in the corresponding area of the opposite cortex [16]. Primary generalized EA was induced by intramuscular injection of bemegride (4-30 mg/kg body weight). Nicotinamide was injected intraperitoneally in doses of 300-1000 mg/kg body weight. The ECoG was recorded on an RM-86 polygraph (Nihon Kohden, Japan). The intensity of lipid peroxidation (LPO) was determined by measuring the concentration of products reacting with 2-thiobarbituric acid (2-TBA) [1] in a suspension of synaptosomes incubated in Krebs–Ringer medium of the following composition (in mM): NaCl — 132, KCl — 5, NaH_2PO_4 — 1.2, $CaCl_2$ — 1.2, $MgCl_2$ — 1.3, glucose — 10, Tris-HCl — 20, pH 7.6 (20°C) at 37°C. LPO was activated by the addition of Fe^{++} ions in the form of $FeCl_2$ (10^{-5} M) and ascorbate (10^{-3} M) to the incubation medium of the synaptosomes.

EXPERIMENTAL RESULTS

Application of 1.2% penicillin solution to two symmetrical areas of the sensomotor cortex simultaneously led to the appearance of EA of the characteristic pattern described previously

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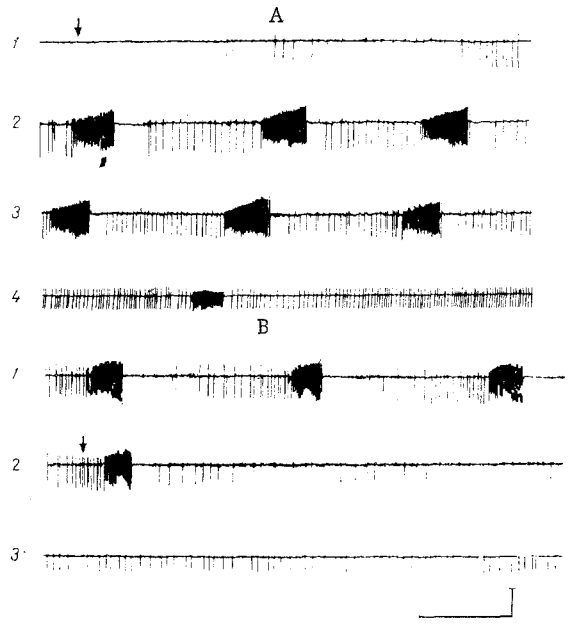

Fig. 1

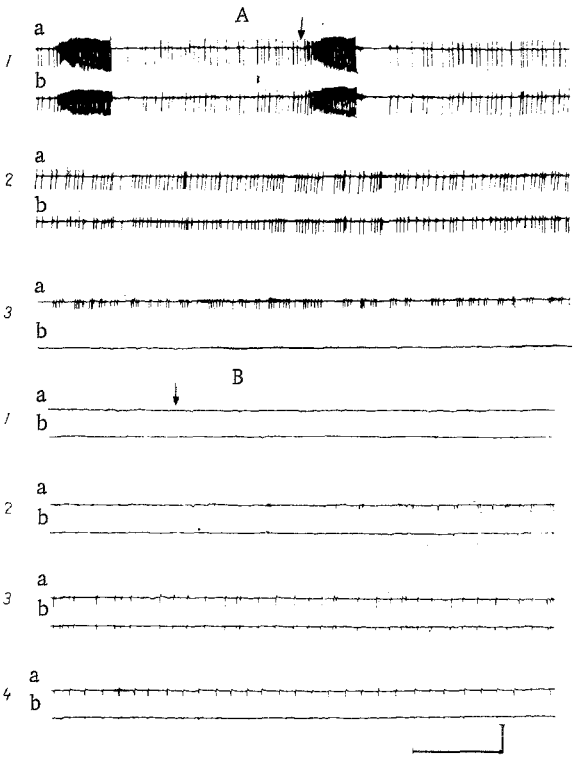

Fig. 2

Fig. 1. Electrical activity in epileptic focus created by penicillin application to rat cortex (A) and activity in similar focus after injection of nicotinamide (B). A: 1) 0-5 min after beginning of recording, 2) 5-10 min, 3) 30-35 min, 4) 85-90 min. Arrow indicates time of penicillin application; B: 1) 30-35 min after beginning of recording, 2) 35-40 min, 3) 85-90 min. Arrow indicates time of injection of nicotinamide (800 mg/kg). Calibration: 1 mV, 30 sec.

Fig. 2. Effect of nicotinamide on electrical activity in primary (a) and "mirror" (b) epileptic foci. A: 1) 30-35 min after beginning of recording, 2) 35-40 min, 3) 85-90 min. Arrow indicates time of injection of nicotinamide (800 mg/kg); B) preliminary (40 min before penicillin application) injection of nicotinamide (500 mg/kg): 1) 0-5 min, 2) 5-10 min, 3) 30-35 min, and 4) 85-90 min after beginning of recording. Arrow indicates time of penicillin application. Calibration: 1 mV, 30 sec.

in these areas [9, 16]. The first interictal discharges (IID) appeared on the ECoG 1-3 min after application. The amplitude of the IID rose rapidly from 200-400 to 700-1000 μV, and 4-8 min after penicillin application the IID began to change into epileptic seizures — ES (Fig. 1A). IID reappeared on the ECoG 0.2-2 min after each ES, and their frequency increased until development of the next ES. The duration of ES was usually 20-30 sec. This order of events on the ECoG was repeated regularly with a mean frequency of 1-2 seizures every 2 min for 1.5-2.5 h, after which the ES disappeared and IID began to be gradually extinguished, so that in a short time they also disappeared from the ECoG. Electrical activity of foci was synchronized in the right and left hemispheres. The life of the focus — from appearance of the first IID to disappearance of the last — averaged about 2.5-3 h.

Injection of nicotinamide (600-800 mg/kg) in the stage of the strongest EA, completely synchronized in the two foci, abruptly weakened EA (Fig. 1B). ES ceased 2-4 min after injection of nicotinamide, and the mean frequency and amplitude of the IID were reduced by 33-50%. ES were not subsequently restored and the amplitude and frequency of IID gradually decreased during the period of existence of the focus. The duration of the period of IID development was not significantly changed compared with the control. The phenomena described took place synchronously in the two foci.

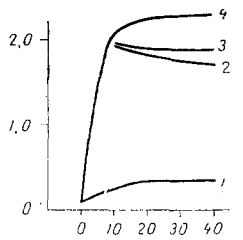

Fig. 3. Effect of nicotinamide on intensity of accumulation of LPO products in suspension of synaptosomes. 1) Fe^{++} + ascorbate + ionol (5×10^{-4} M); 2) Fe^{++} + ascorbate + nicotinamide (10^{-5} M); 3) Fe^{++} + ascorbate + nicotinamide (10^{-8} M); 4) Fe^{++} + ascorbate. Abscissa, time of incubation of synaptosomes (in min); ordinate, concentration of products reacting with TBA (in nmoles/mg protein).

In the same way, when nicotinamide was injected in the same doses in the initial period of activity of the EA focus (after 3-5 seizures had occurred from the time of application of penicillin) disappearance of ES and a progressive decrease in the frequency and amplitude of IID were observed. Later, on average 1.5 h after injection of nicotinamide, ES reappeared on the ECoG. A similar result was obtained previously with strychnine-induced foci of EA in cats [5].

In the experiments of series II, the effect of nicotinamide was studied on the intensity of EA in a primary (determinant [3]) focus of EA created by application of 1.2% penicillin solution to one hemisphere, and in the dependent, "mirror" focus. Injection of nicotinamide in doses of 800-1000 mg/kg in the stage of maximal EA, synchronized in the two foci, caused weakening of EA both in the primary and in the "mirror" focus after 1-3 min (Fig. 2A).

Preliminary injection of nicotinamide in a dose of 500 mg/kg 40 min before penicillin application prevented the development of ES in the animal (Fig. 2B).

Primary generalized EA was induced in the animals by intramuscular injections of 0.5% bemegride solution. Injection of bemegride in doses of over 10 mg/kg led to the appearance of violent motor activity in the animal accompanied by a characteristic ECoG picture [16]. After injection of lethal doses of bemegride (over 15-18 mg/kg) the animals died, usually at the height of a seizure. Injection of nicotinamide in the stage of the most clearly defined and stable picture of EA evoked by injection of sublethal doses of bemegride (10-15 mg/kg), even in massive doses (100-1200 mg/kg) did not affect the intensity of generalized EA. Nicotinamide affected neither amplitude nor shape nor frequency of the seizure discharges and did not prevent death of the animal from lethal doses of bemegride. The absence of any effect of nicotinamide on generalized metrazol or audiogenic convulsions was demonstrated previously. Meanwhile, preliminary injection of nicotinamide in a dose of 500 mg/kg 40 min before injection of bemegride in a dose of 10-15 mg/kg lengthened by 2.5 times the latent period of appearance of the first ES on the ECoG, and increased correspondingly the threshold seizure dose of bemegride.

The results thus indicate that nicotinamide, in the doses tested, has marked antiepileptic activity. This effect can be explained by interaction of nicotinamide with benzodiazepine receptors, leading to subsequent activation of GABA receptors [5, 6, 11-15]. Data showing the similarity between the effects of diazepam and nicotinamide on mediator metabolism in different parts of the rat brain [10] are indirect evidence in support of this hypothesis. However, there is another possible explanation of the antiepileptic action of nicotinamide.

Certain synthetic derivatives of nicotinamide are known to possess marked antioxidative activity [2]. This is an interesting fact because the writers showed previously that the appearance of EA in the cerebral cortex of animals is accompanied by a sharp increase in the intensity of LPO in that structure; preliminary administration of antioxidants prevented the intensification of LPO and sharply depressed EA [4, 7, 8]. The question arises whether the antiepileptic action of nicotinamide may be due to its ability to reduce the intensity of LPO in neuron membranes.

To test this hypothesis, the effect of nicotinamide on the intensity of accumulation of LPO products in a suspension of synaptosomes was studied. Addition of LPO activators (Fe^{++} ions and ascorbate) to the incubation medium led to a sharp increase in the concentration of products reacting with TBA in the system (Fig. 3). This increase could be prevented by preliminary (5 min before addition of the LPO activators) addition of antioxidants — EDTA (5×10^{-3} M) or ionol (5×10^{-4} M) — to the suspension. Like these known antioxidants, nicotinamide (10^{-8} M and 10^{-5} M) lowered the intensity of accumulation of LPO products in the suspension of synaptosomes (Fig. 3). Consequently, nicotinamide exhibited definite antioxidative activity in this system.

This suggests that the fact that nicotinamide possesses antiepileptic activity may be connected to some degree with its antioxidative properties. The possibility cannot be ruled out that the antiepileptic action of nicotinamide is mediated in the body through its metabolic products which possess antioxidative activity.

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