Psychoregulatory Role of Nicotinamide

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Nicotinamide, which is regarded as an endogenous ligand of benzodiazepine (BZ) receptors and which is capable of inhibiting competitively the specific binding of benzodiazepines in the brain [25], displayed well-defined sedative [8] and anticonvulsive and stress-protecting properties in experimental studies [2, 11, 13]. Nicotinamide and nicotinic acid deficiency in the body occurs in various nervous and mental disorders, including neuroses and depressions [12, 15].

Here we report the findings of our experimental and clinical studies designed to assess the psychotropic activity of nicotinamide with a view to elucidating the prospects for its use in a new capacity - for the treatment of psychoneurotic disorders.

MATERIALS AND METHODS

For the experimental study, male rats and mice weighing 250-300 and 22-26 g, respectively, were used. They were given an intraperitoneal injection of nicotinamide at 250-500 mg/kg body weight 30 to 40 min before the tests. The anxiolytic effect of nicotinamide was evaluated in a conflict test [7, 8]. The involvement of GABA-ergic mechanisms in mediating this effect was assessed using the GABA agonist calcium valproate (200 mg/kg) and the GABA antagonists bicuculline (1 mg/kg) and picrotoxin (5 mg/kg). For demonstrating the role of BZ receptors, their specific antagonist Ro 15-1788 was used, administered at 10 mg/kg 10 min before the testing. Radioligand affinity for BZ receptors was tested using the sum of fractions of rat hippocampal membranes as described previously [24]. Assays for the nootropic (antihypoxic and antiamnestic) activities of nicotinamide were carried out on animals with hypoxia of various origin and those with shock-induced amnesia. Acute hypobaric hypoxia was produced in a pressure chamber at an "altitude" of 10.5-11 km, to which the animals were elevated at an average rate of 50 m/sec. Normobaric hypoxia (3% oxygen and 97% nitrogen) and hemic (methemoglobin) hypoxias were produced as described elsewhere [10]. The antiamnestic activity of nicotinamide was assayed by its ability to eliminate the amnesia caused by maximal electroshock in animals that had developed a passive avoidance response [22], and was compared to those of classic benzodiazepine tranquilizers, including diazepam [8].

The clinical study was carried out in a hospital setting on patients with neurasthenia (20 men and women), delirium tremens (8 men), alcohol withdrawal (15 men), or epilepsy (10 men and women). The patients, who ranged in age from 20 to 56 years and had been ill for 6-12 months to 2-3 years, were given nicotinamide four times daily, either orally in 0.025 g tablets (4 to 8 tablets) or by injection from 2 ml ampoules containing a 5% nicotinamide solution (2-4 ampoules). The efficacy of this treatment in the various groups was evaluated in comparison with the minor effects produced in the same patients previously by other psychotropic drugs such as diazepam (2-5 mg/kg), phenazepam (0.5-1 mg/kg),

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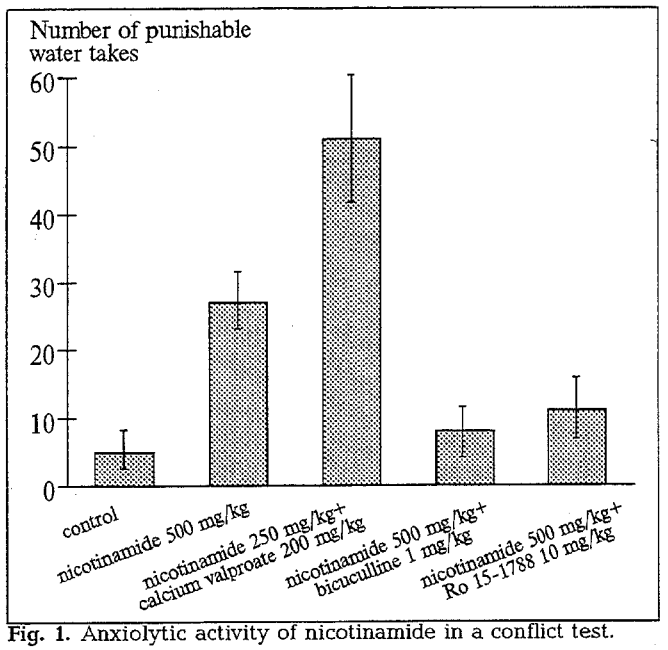

Fig. 1. Anxiolytic activity of nicotinamide in a conflict test.

piracetam (250-500 mg/kg), and meclofenoxate (100-200 mg/kg).

The results were analyzed statistically by Fisher's test. Differences between the groups were considered to be significant at $p<0.05$ and highly significant at $p<0.01$ [3].

RESULTS

Evidence for the ability of nicotinamide to act as a tranquilizer was provided by tests of its anxiolytic activity. These demonstrated marked antianxiety effects of nicotinamide manifested, in particular, in an increased number of water takes by the animals despite painful electrical stimulation (Fig. 1). The anxiolytic effect was decreased by the GABA antagonists bicuculline and picrotoxin and increased by the GABA agonist calcium valproate, which indicates that a considerable role in mediating this effect is played by the GABA-ergic system. The anxiolytic effect of nicotinamide, like that of diazepam and other benzodiazepines, could be abolished by the BZ receptor antagonist Ro 15-1788. These findings suggest that both BZ and GABA receptors are instrumental in mediating the tranquilizing action of nicotinamide. In another study, evidence was provided that a major mechanism responsible for the GABA-potentiating action of benzodiazepines, nicotinamide, inosine, and harmane is Ca-dependent modulation of the chloride channels coupled to $GABA_A$ receptors [1]. The affinity of a substance for BZ receptors is a predictor of its properties associated with anxiolytic activity and resulting from its interaction with different subtypes of these receptors. In our study of the nicotinamide interaction with BZ receptors of the brain, an IC_{50} value of 7.7 mmol/liter was obtained, which agrees with the values published in the literature [25, 28]. In general, nicotinamide has shown itself as an agonist of GABA and BZ receptors of the central type *in vivo* and as a substance possessing considerable affinity for the specific ^{3}H-diazepam binding sites *in vitro*, although the question of whether nicotinamide is indeed a GABA and BZ receptor agonist *in vivo* has been widely debated [20, 21, 25]. An argument against the ability of nicotinamide to act as an endogenous ligand of BZ receptors is its relatively low affinity for these receptors as compared, for example, with β-carbolines. In our view, however, there are no grounds for ruling out the local presence of nicotinamide, as well as of inosine, hypoxanthine, and adenosine, in the synaptic area of brain structures in concentrations sufficient for the occupation of a proportion of BZ receptors. Considering the actual affinities of these substances, it can be said that their concentrations required for the occupancy of 25-30% of BZ receptors should be approximately 10^{-4} mmol/liter, which seems realistic. This probably explains why nicotinamide, which is a natural endogenous cell component, exerts its tranquilizing effect.

Important results came from tests using pairs of nicotinamide-treated animals displaying aggressive reactions toward each other elicited by motives similar to natural ones. Nicotinamide treatment made the animals less aggressive and led to a decreased number of fights between them on an electrifiable grid floor (the ED_{50} of nicotinamide for this latter effect was 500 mg/kg).

Of special note are the antihypoxic and antiamnestic properties of nicotinamide revealed in our study. It has been shown for the first time that

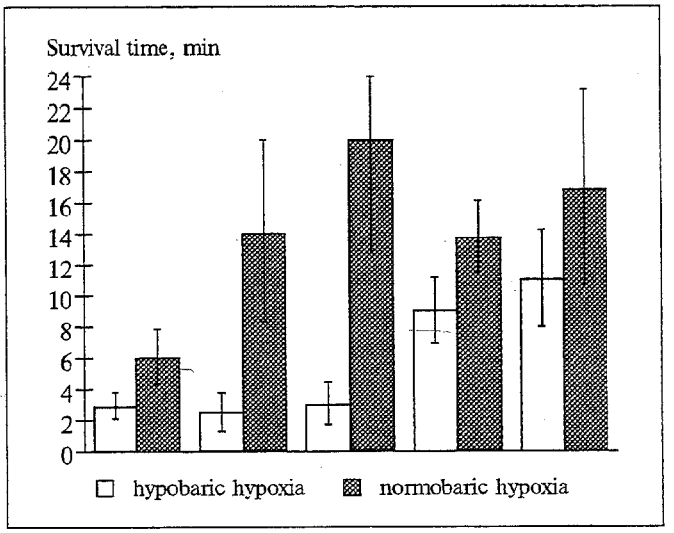

Fig. 2. Antihypoxic activity of nicotinamide compared to that of piracetam.

nicotinamide in the doses used is capable of affording protection to animals with acute hypoxic states of various origin. For example, nicotinamide increased twofold or more the survival time of animals with acute hypobaric hypoxia induced in a pressure chamber. Under conditions of normobaric hypoxia in an atmosphere with low partial oxygen pressure, nicotinamide was several times more efficient than piracetam in protecting animals from death (Fig. 2). It also increased, by 120-150%, the survival of animals with hemic hypoxia. Piracetam, which was used for comparison, failed to contribute significantly to the survival of animals with hypoxia induced in the pressure chamber. These findings agree with those reported by other authors who found that neither piracetam nor its analog aniracetam could prevent the death of mice from hypobaric hypoxia [23, 27], and that piracetam was also ineffective in normobaric hypoxia [18], although there is evidence that piracetam is particularly active in the latter type of hypoxia [16, 17].

In our view, the antihypoxic activity of nicotinamide may be explained in part by its ability to make the brain, and in particular its cortical structures, more resistant to oxygen deficiency. This view is supported by the reported ability of many nootropic agents to improve metabolism in nerve tissues, primarily cortical structures [20, 26].

Although important, animal models of hypoxia have a lower predictive value for evaluating the actions of nootropic agents than do those of amnesia [9]. In animals with amnesia induced by maximal electroshock, nicotinamide was found to exert an antiamnestic effect (Fig. 3), regardless of whether the animals received it before or after the shock. Both in normal animals and those with retrograde amnesia, nicotinamide improved the learning process as well as memory at the fixation, consolidation, and reproduction stages. It is significant that it influenced predominantly memory consolidation, which is believed [4] to be of key importance in the system controlling memory mechanisms.

The effects of piracetam on the learning process did not differ appreciably from those described for this compound by other authors [17, 23] and were similar to the effects of nicotinamide but not to those of more potent nootropic agents such as meclofenoxate and pyritinol. A moderate antiamnestic activity of piracetam was reported by Rakhmankulova *et al.* [19], who pointed out that it only slightly improved the process of passive avoidance response learning by animals with shock-induced amnesia. In our study, the benzodiazepine derivatives diazepam and phenazepam, unlike nicotinamide, not only failed to prevent the development of retrograde amnesia, but even decreased the animals' capacity for reproducing the learned passive avoidance response, i.e., it exerted an amnestic effect.

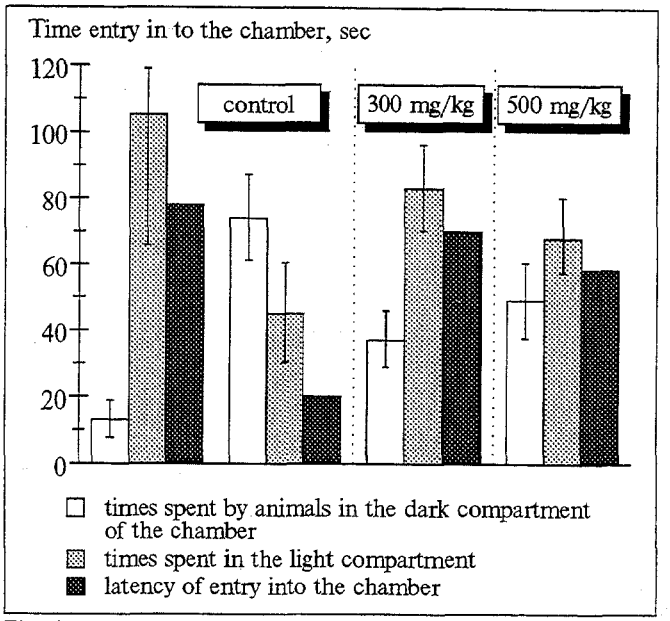

Fig. 3. Antiamnestic activity of nicotinamide compared to that of piracetam in amnesic animals, as assessed by their passive avoidance response.

The current concepts of how nootropic agents act attach particular importance to the membrane functions of the cell. Piracetam and oxiracetam, for example, have been shown to stimulate phosphatidylcholine and phosphatidylethanolamine metabolism by activating phospholipases A_1 and A_2, which indicates that they can stabilize and maintain structural and functional characteristics of the cell membrane [23]. Membrane-mediated mechanisms appear to underlie the actions of nootropic drugs on memory and probably also determine the antiamnestic effects of nicotinamide. This contention is supported by the membranotropism of nicotinamide, which has been shown capable of modifying the phospholipid com-

TABLE 1. Effectiveness of Psychopharmacotherapy with Nicotinamide

Mental disorder	№ of patients	Proportion of patients with marked improvement	Significance of difference from the control group
Neurasthenia	20	75%	$p<0.01$
Delirium tremens	8	60%	$p<0.05$
Alcohol withdrawal syndrome	15	53.3%	$p<0.05$
Epileptic seizures	10	80%	$p<0.01$

position of membranes and of preventing free-radical processes [5, 6, 14]. Nicotinamide appears to be able to cause certain conformational changes in protein molecules and to optimize the formation, consolidation, and storage of memory traces.

Nicotinamide treatment of 20 patients with neurasthenia accompanied by hyposthenia led to considerable improvements in 16 of them, including 4 who had previously been unsuccessfully treated with tranquilizers. The improvements, which were evaluated by both subjective and objective indicators, occurred after 20 days of hospital treatment.

Patients with alcoholism (8 with delirium tremens and 15 with alcohol withdrawal syndrome) were receiving nicotinamide along with other nonspecific therapies (vitamins, detoxification, etc.). The use of nicotinamide resulted in much less pronounced signs of postpsychotic asthenia as compared to the use of specific antialcoholic drugs, so that the treatment could be terminated earlier. For instance, the condition of patient N.S., a man aged 35 years who had been admitted with striking mental abnormalities due to alcohol intoxication, improved appreciably after 5 days of daily nicotinamide injections, as compared to 20-30 days when the conventional therapeutic regimen was used.

In most patients with acute delirium tremens, nicotinamide treatment decreased anxiety, tremor, withdrawal symptoms, and postpsychotic asthenia and also improved attention and recall. In the nicotinamide-treated group with the alcohol withdrawal syndrome (n=15), accelerated disappearance of asthenic/neurotic manifestations was observed (in 8 cases), as were a considerable diminution of somatic neurological disorders and memory improvement (in 10 cases).

In the group with epilepsy (n=10), epileptic seizures combined with mental disturbances, dysphoria, and manifestations of hostility and aggressiveness; the frequency of seizures and the severity of affective disorders were relatively stable. Treatment with nicotinamide alone, without any additional anticonvulsants, led to significantly less frequent seizures of the *grand mal* type and to the disappearance of dysphoria in 9 of the 10 patients.

For example, patient G.S., a 30-year-old woman with a 4-year history of epilepsy, was admitted because of increased frequency of seizures (previously she had been treated with anticonvulsants, tranquilizers, and antidepressants). In the hospital, her treatment with nicotinamide alone during the first 20 days resulted in less frequent seizures of the *petit mal* type and in less marked dysphoria; on the 21st day anticonvulsants were added to nicotinamide, and she was discharged 10 days later in a satisfactory condition – with instructions to continue the combined treatment.

It should be stressed that prolonged nicotinamide treatment had marked effects in protecting the patients from recurrent epileptic seizures and dysphoric disturbances. Our studies and those by other authors [13] indicate that nicotinamide suppresses the epileptic focus through activation of the GABA-BZ receptor complex and through inhibitory control of GABA, which supports the concept that antiepileptic properties are inherent in endogenous nicotinamide and that this compound participates in regulating the generation of electrical potentials in the brain.

In conclusion, the present studies indicate that nicotinamide is capable of counteracting emotional instability, tension, and anxiety and of enhancing the body's resistance to highly adverse factors, and that it may therefore be used to treat psychoneurotic disorders of various origin. Unlike tranquilizers and antidepressants, it exhibits nootropic rather than anamnestic activity (improves attention and memory). It also exerts anticonvulsive and antiaggressive effects. All this makes nicotinamide a promising agent for use in the management of epilepsy and convulsions as well as of acute alcoholic delirium and alcohol withdrawal.

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Effect of Phenobarbital Pretreatment on the Toxicity of GABA-lytic Agents in Mice

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Barbiturates are known to increase the resistance of animals to toxic effect of phosphoroorganic compounds (POS) [5, 10]. The effect is connected with a modulation of the activity of the monooxygenase system, blood and liver carboxyl esterases, and other detoxication systems [4, 6, 7, 11].

It may be speculated that the advance administration of inducers of the biological defense systems could change the sensitivity of animals to GABA-lytic agents.

In order to elucidate this problem, in the present study we assessed the toxicity of picrotoxin, bicuculline, and 3-mercaptopropionic acid (3-MPA) in male white mice pretreated three times with phenobarbital and benzonal. We also studied the antidote efficiency of diazepam in picrotoxin and bicuculline toxicity under conditions of phenobarbital-mediated modulation of detoxication systems.

MATERIALS AND METHODS

Mature male white mice weighing 26-30 g were injected intraperitoneally with sodium phenobarbital or benzonal (40 mg per kg body weight) daily for 3 days. The toxicity of picrotoxin, bicuculline, and 3-MPA was assayed 24 hours after the last injection. Picrotoxin and bicuculline were suspended in saline with Tween 80. 3-MPA was dissolved in saline. The agents were introduced via the intraperitoneal route. Diazepam was administered intraperitoneally in a dose of 5 mg per kg 10, 30, 90, and 180 min before the GABA-lytic agents. All substances used in the study were purchased from Sigma. At least six animals per dose were used and at least five doses were tested in the course of the determination of toxicity. LD_{50} was calculated using regression analysis with the method of least squares. The reliability of the differences was evaluated using Student's *t* test.

RESULTS

Table 1 presents the data regarding the toxicity of picrotoxin, bicuculline, and 3-MPA in the mice 24 hours after they had received the third injection of phenobarbital and benzonal. Under these conditions the resistance of the animals to picrotoxin and bicuculline reliably increased. For instance, in the mice pretreated with benzonal, the resistance rose by

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