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MECHANISM OF THE TRANQUILIZING ACTION OF SOME ELECTRONIC STRUCTURAL ANALOGS OF NICOTINAMIDE

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Nicotinamide, hypoxanthines, β-carbolines, and various other endogenous brain compounds are probable endogenous ligands of benzodiazepine receptors (BDR) [11, 12, 14, 15]. It has been shown that nicotinamide possesses certain pharmacologic properties on the basis of which it can be regarded as a tranquilizer [1, 2, 4, 8, 9]. It is interesting in this connection to search for compounds with tranqilizing activity, superior to that of nicotinamide, among its analogs (amides of aminonicotinic, hydroxynicotinic, and hydroxyisonicotinic acids, esters of nicotinic acid) with definite electronic structural similarity with tranquilizers of the benzodiazepine series [7].

With this aim, and using a model of a conflict situation and the technique of radioligand analysis, the writers investigated two original compounds (coded NMF and AzN), which are ethyl esters of nicotinic acid. Nicotinamide itself and inosine, another hypothetical ligand of BDR, were used as substances for comparison.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-230 g. The anxiolytic effect of nicotinamide (250-500 mg/kg), inosine (500 mg/kg), NMF, and AzN (20 mg/kg) was studied under conditions of a conflict situation [3, 5]; the substances were injected intraperitoneally 30-40 min before testing. Participation of GABA-ergic mechanisms in the realization of the anxiolytic action of the compounds was demonstrated by the use of specific analyzers: calcium valproate (200 mg/kg) and bicuculline (1 mg/kg), which were injected 40 and 5 min respectively before the experiment. To discover the role of central BDR, the specific antagonist of benzodiazepine receptors Ro 15-1788 was used in a dose of 10 mg/kg, given 10 min before testing. The effect of the test substances, in concentrations

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TABLE 1. Effect of Nicotinamide, Inosine NMF, and AzN on Uptake of ^{3}H-GABA, ^{14}C-Glutamate, and ^{3}H-Serotonin (10 μM, in % of control) by Coarse Synaptosomal Fraction (M ± m, P = 0.05)

Compound	GABA, μm		Glutamate, μm		Serotonin, μm	
	50	500	50	500	50	500
Nicotinamide	100±12	96±12	120±20	94±12	105±14	93±12
Inosine	118±15	120±15	115±14	91±10	127±18	98±12
NMF	103±12	86±10	72±11	66±10	125±18	105±12
AzN	97±11	84±10	103±12	117±15	105±12	75±10

Legend. Results of five to seven measurements are shown. Control: 3 nmoles of bound GABA and glutamate and 0.05 nmole of bound serotonin during 20 min per milligram protein.

TABLE 2. Effect of Nicotinamide and its Electronic Structural Analogs NMF and AzN on Specific Binding of ^{3}H-Diazepam by Rat Hippocampal Membranes (M ± m)

Compound	Ki_{50}, mM
Nicotinamide	7,6 1,3
NMF	3,1 0,5
AzN	0,9 0,2

Legend. Results of five or six experiments shown. Difference between values is significant by Student's test at the P < 0.02 level.

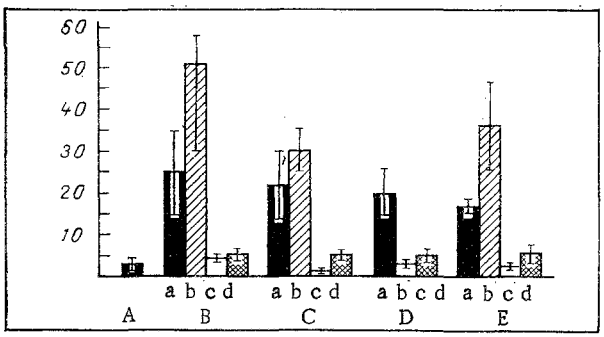

Fig. 1. Effectiveness of the anxiolytic action of nicotinamide (B), NMF (C), AzN (D), and inosine (E). A) Control; a) test compound, b, c, d) combination of test compound with calcium valproate (200 mg/kg), bicuculline (1 mg/kg), and Ro 15-1788 (10 mg/kg) respectively. Nicotinamide 250 mg/kg, NMF 20 mg/kg, AzN mg/kg, inosine 500 mg/kg. Ordinate, number of punishments for taking water.

of 50 and 500 μM, on uptake of ^{3}H-GABA, ^{14}C-glutamate, and ^{3}H-serotonin by the coarse synaptosomal fraction, isolated from rat brain, was studied by the method of Snyder and Coyle in the modification in [6, 10]. For radioligand study of affinity for BDR, a total fraction of rat hippocampal membranes was used a in [13]. The incubation mixture contained, in a volume of 0.5 ml: ^{3}H-diazepam (Amersham Corporation, England, 89 Ci/mmole) in a concentration

of 5 nM; membranes 40-80 μg, 100 mM NaCl, 50 mM Na,K-phosphate buffer, pH 7.4. To determine nonspecific binding 1 μM diazepam was used.

EXPERIMENTAL RESULTS

Experiments on a model of a conflict situation showed that nicotinamide, its analogs NMF and AzN, and inosine, like preparations of the benzodiazepine series (diazepam), had an anxiolytic action. This was manifested as an increase in the number of visits to the drinking bowl and the number of times of taking water, even though the rat received a painful stimulus on the establishment of a stable drinking reflex to the situation. It should be emphasized also that the effects of NMF and AzN appeared in response to smaller (by more than an order of magnitude) doses compared with those of nicotinamide and inosine. Analysis of the results showed that an important role in the realization of the anxiolytic action of the substances was played by the GABA-BDR complex, as is shown by the weakening of this effect under the influence of bicuculline and thiosemicarbazide and its potentiation by the action of calcium valproate. Like diazepam and the related benzodiazepines, the anxiolytic action of nicotinamide was prevented by the specific BDR antagonist Ro 15-1788. The anxiolytic action of the structural analogs of nicotinamide, NMF and AzN, was prevented in the same way. Antagonism between inosine and Ro 15-1788 with respect to its anxiolytic action was less marked (Fig. 1). When the effects of the analyzers on those of nicotinamide and its analogs were compared it was noted that bicuculline, which blocks GABA-ergic receptors, weakened the anxiolytic action of NMF and AzN much more effectively ($P < 0.05$) than nicotinamide. The results also showed that whereas the anxiolytic action of nictoinamide is abolished by bicuculline and Ro 15-1788 equally, the action of these two analyzers on the nicotinamide analogs differed, for the specific antagonist of GABA receptors was more effective than the BDR antagonist. The results are evidence of a possible role not only of benzodiazepine, but also of GABA receptors, in the realization of the anxiolytic action of nicotinamide analogs, and this would explain the higher activity of these substances compared with nicotinamide.

Investigation of the uptake of labeled GABA, glutamate, and serotonin by the coarse synaptosomal fraction showed that the substances studied, in a concentration of 50 μM, had no inhibitory action on the uptake of these mediators, with the exception of NMF, which inhibited glutamate uptake by 30%. With an increase in the concentration of these substances to 500 μM the process of inhibition of accumulation of the labeled mediators was stimulated a little, and was observed mainly with nicotinamide analogs (Table 1). It can be concluded from analysis of the results that the mechanism of the tranquilizing action is evidently not determined by the influence of the test substances on the process of reuptake of the mediators by synaptosomes, for the effects of inhibition of uptake were observed only in high concentrations, and were evidently connected with the nonspecific membranotropic action [6].

Comparison of the ability of nicotinamide, NMF, and AzN to inhibit specific binding of ^{3}H-diazepam with the membrane fraction of the hippocampus showed (Table 2) that the electronic structural analogs of nicotinamide bound more actively with BDR *in vitro* ($P < 0.02$). The values of Ki_{50} obtained for nicotinamide agreed with those published previously [14] for this compound and inosine. On the whole, the nicotinamide analogs which we investigated behaved *in vivo* as agonists of GABA-receptors and BDR of central type, which have significantly greater affinity than nicotinamide for specific binding sites of ^{3}H-diazepam *in vitro*. The tranquilizing action revealed thus is realized, not at the level of regulation of mediator concentrations (GABA, glutamate, serotonin), but due to direct interaction between the compounds studied and the GABA-benzodiazepine receptor complex. The fact that according to the results of pharmacologic and radiologic analysis, NMF and AzN are more active tranquilizers than nicotinamide, can also be explained on the grounds that nicotinamide, as an active component of the cell, is metabolized much more rapidly than its electronic structural analogs.

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EFFECT OF A SINGLE INJECTION OF ETHANOL ON PERMEABILITY OF THE BLOOD-BRAIN BARRIER FOR ^{14}C-TYROSINE, ^{14}C-DOPA, AND HORSERADISH PEROXIDASE

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When administered peripherally ethanol easily passes through the blood-brain barrier (BBB) and enters the brain [10]. It has also been shown that ethanol disturbs the entry of products taking part in metabolism into the brain and, in particular, amino acids [6, 12], which pass through the BBB with the participation of specific mechanisms of active transport [13]. It can accordingly be concluded that the activity of at least some of the eight known [13] systems for the transport of low-molecular-weight metabolites to the brain is modified by ethanol. The mechanism of nonspecific transport of high-molecular weight compounds of protein type into the brain (the discovery of interendothelial tight junctions, vesicular transport, endothelial tubules, diffusion through the endothelial cytoplasm), reflecting the state of the barrier function of the BBB, are normally at a low functional level, but they may be activated under the influence of various stress-inducing and injurious factors [4, 8-14], which may include the intake of ethanol.

The aim of this investigation was to make a comparative study of the effect of a single intake of ethanol on some mechanisms of specific and nonspecific transport of materials across the BBB.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 300-350 g. Ethanol was injected intraperitoneally in doses of 1, 2, and 4 g/kg body weight. After 60 min, under ether anesthesia, ^{14}C-L-tyrosine or ^{14}C-L-dopa (specific radioactivity 492 and 5.4 kCi/mmole respectively) was injected into the femoral vein in a dose of 5 μCi, dissolved in 0.5 ml of physiological saline. In the experiments with ^{14}C-dopa, to prevent its peripheral metabolism, carbidops (α-methyldopa hydrazine, an inhibitor of dopa-decarboxylase [7]) was injected intraperitoneally in a dose of 80 mg/kg 75 min before the indicator. Immediately

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