NOOTROPIC AND ANXIOLYTIC PROPERTIES OF ENDOGENOUS LIGANDS OF BENZODIAZEPINE RECEPTORS AND THEIR STRUCTURAL ANALOGS

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Nicotinamide (NAM) and inosine are nowadays regarded as endogenous ligands of benzodiazepine receptors [10, 11]. They are known to be able to bind with specific binding sites of benzodiazepines [10, 14] and to induce effects similar to those of drugs of the benzodiazepine series. In particular, NAM has experimental anticonvulsant, anticonflict, and antiaggressive activity, and inhibits motor activity [7, 10]; an antimetrazol action has been demonstrated for inosine [12].

Considering modern views on the GABA-benzodiazepine receptor complex [8, 9, 13] and data showing that benzodiazepines have antihypoxic properties [5], the object of this investigation was to study the nootropic and anxiolytic effects of inosine, NAM, and its new structural analogs — NMF and AZN, and also to study the role of the GABA-ergic system in the realization of the anxiolytic action of these substances.

EXPERIMENTAL METHOD

The investigation was carried out on noninbred male albino mice weighing 18-26 g and rats weighing 180-240 g. Antihypoxic activity was studied on a model of hypobaric hypoxia in a push–pull pressure chamber ("altitude" 10,500-11,000 m, mean rate of ascent V = 50 m/sec). Antiamnesic activity was estimated by the ability of the substances to abolish amnesia induced by maximal electroshock under the conditions of the passive avoidance technique in mice [1]. Amnesia was created by applying maximal electroshock by means of corneal electrodes immediately after learning, to obliterate the memory trace. The anxiolytic effect was studied by the conflicting situation technique, created in rats by conflict between two opposite motivations — defensive and food-getting [3]. As analyzers of the GABA-ergic system we used calcium valproate (200 mg/kg, intraperitoneally, 30 min before the experiment) and bicuculline (1 mg/kg, subcutaneously, 5 min before the experiment). The test substances, namely NAM (250-2000 mg/kg), inosine (500-2000 mg/kg), NMF, and AZN (10-50 mg/kg), and diazepam (10 mg/kg) were injected intraperitoneally 30 min before the experiments. For statistical analysis of the results mean values and their confidence limits at a level of significance of P = 0.05 were calculated.

EXPERIMENTAL RESULTS

The study of antihypoxic activity shows that all substances tested were able to substantially increase the duration of survival of the mice exposed to acute hypoxia (Table 1). The new compounds NMF and AZN had the greatest activity under conditions of hypobaric hypoxia: In a dose of 10 mg/kg they doubled the survival time of the mice. Diazepam, used as the reference substance, had approximately the same action. The activity of NAM and inosine was much weaker than that of their structural analogs. Their antihypoxic effects were manifested only in doses of 250-1000 mg/kg. Evidence of the moderate antihypoxic activity of inosine is given in [6], in which it is stated that in a dose of 250 mg/kg the protective effect of inosine was 139%. It follows from the work of Vladimirov and Archakov [2] that one aspect of the protective action of inosine under conditions of hypoxia is its ability to inhibit lipid peroxidation, and thus to restore structural and energy metabolism in the cell. A definite role in the mechanism of the antihypoxic action of these substances, like that of

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TABLE 1. Study of Survival of Mice (in min) During Hypobaric Hypoxia under the Influence of Diazepam, NAM, Inosine, NMF, and AZN

Substance	Dose, mg/kg	Effect, min
	Control	3,2 (1,0—7,0)
Diazepam	5	18,6 (2,5—30,0)
	10	24,4 (10,0—30,0)
NAM	Control	0,57 (0,22—2,0)
	500	6,3 (0,9—30,0)
	1000	6,8 (1,0—30,0)
Inosine	Control	1,4 (0,45—3,0)
	250	11,3 (1,0—30,0)
	500	22,3 (4,0—30,0)
NMF	Control	2,25 (0,5—5,5)
	10	5,7 (1,0—17,0)
	50	22,6 (8,0—30,0)
AZN	Control	3,1 (0,5—6,0)
	10	7,6 (1,0—30,0)
	50	16,8 (1,0—30,0)

TABLE 2. Comparative Action of Inosine, NAM, NMF, and Diazepam on Behavior of Rats in Conflict Situation

Substance	Dose, mg/kg	Number of visits to drinking bowl	Number of times water was taken	Motor activity
Control	Starch suspension	6,3 (4,1—8,9)	3,3 (1,6—5 ,9)	35,6 (26,5—39,5)
Diazepam	10	10,4 (8,1—19,7)	19 (12,7—24,9)	9,4 (5,6—13,6)
NAM	125	7,7 (4,3—11,1)	5,8 (3,9—7,7)	10,4 (7,6—13,2)
	250	3,4 (0,6—6,2)	24,5 (14,0—35,0)	15,1 (8,2—22,0)
Inosine	250	11 (6,6—15,4)	3,7 (2,8—4,6)	18,5 (15,1—21,9)
	500	7,5 (4,9—10,1)	15,5 (11,8—19,2)	9,2 (3,3—15,1)
NMF	10	8,6 (3,9—15,1)	18 (14,2—25,8)	29,7 (12,1—35,9)
	20	26,3 (20,2—38,8)	22,7 (14,3—35,8)	12,7 (8,9—18,1)

diazepam, may be played by an increase in resistance of the brain and, in particular, of cortical structures to oxygen deficiency [5]. It can be tentatively suggested that one of these mechanisms participates in the realization of the antihypoxic effect of the new compounds.

Besides their antihypoxic effect, the substances tested also had antiamnesic activity, studied by the passive avoidance method. Their effect was expressed as lengthening of the latent period of visiting the darkened compartment where previously (24 h beforehand) they had received a single training painful stimulus followed by electroshock. The latent period of visiting the darkened compartment by the control animals after electroshock, obliterating the memory trace, was 33.5 ± 13.2 sec. Inosine increased this time to 46.5 ± 11.0 sec, NAM to 66.5 ± 24.4 sec, and NMF to 80 ± 24.4 sec. The antiamnesic activity of these substances thus increases in the order: inosine < NAM < NMF.

Experiments in a conflict situation showed that benzodiazepine receptor ligands inosine and NAM, and also the structural analog of NAM — NMF — possess marked anxiolytic activity. Like drugs of the benzodiazepine series, notably diazepam, these substances increase the number of visits made by the animals to the drinking bowl and the number of times of drinking water, despite the painful electrical stimulation received while so doing (Table 2). The anxiolytic effect of inosine and NAM was inhibited in doses of 250-500 mg/kg, whereas the effect of NMF, the structural analog of NAM, was observed in much smaller doses (by more than one order of magnitude), namely 10-20 mg/kg. The anxiolytic activity of NMF is similar to that of diazepam and stronger than that of chlordiazepoxide.

To study the possible participation of GABA-ergic mechanisms in the realization of the anxiolytic effect, the action of the substances was studied in conjunction with bicuculline (which blocks GABA-ergic receptors) and calcium valproate (which raises the brain GABA concentration). The experiments showed that calcium valproate increases the effect of inosine, NAM, and NMF, whereas bicuculline reduces the action of these substances in a conflict situation. The anxiolytic action of NMF was abolished by a much greater degree than the effects

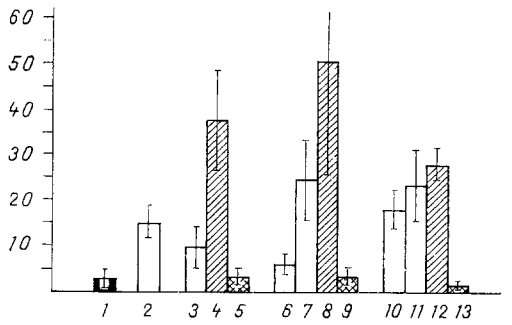

Fig. 1. Effect of NAM, inosine, NMF, and their combinations with calcium valproate and bicuculline on behavior of rats in a conflict situation. Ordinate, number of punished visits to drinking bowl for water. 1) Control; 2) calcium valproate 200 mg/kg; 3-5) inosine; 3) 300 mg/kg; 4) inosine 300 mg/kg + calcium valproate 200 mg/kg; 5) inosine 500 mg/kg + bicuculline 1 mg/kg; 6-9) NAM; 6) 125 mg/kg; 7) 250 mg/kg; 8) NAM 125 mg/kg + calcium valproate 200 mg/kg; 9) NAM 250 mg/kg + bicuculline 1 mg/kg; 10-13) NMF; 10) 10 mg/kg; 11) 20 mg/kg; 12) NMF 10 mg/kg + calcium valproate 200 mg/kg; 13) NMF 20 mg/kg + bicuculline 1 mg/kg.

of inosine and NAM by bicuculline, whereas calcium valproate potentiated the action of inosine and NAM more strongly (Fig. 1). These results indicate that the GABA-ergic system participates in the realization of the anxiolytic action of inosine, NAM, and NMF.

All the substances studied thus possess definite antihypoxic, antiamnesic, and anxiolytic properties. The fact that endogenous ligands of benzodiazepine receptors inosine and NAM, and also the new compounds NMF and AZN, have antihypoxic and antiamnesic effects indicates that these substances possess nootropic activity, which distinguishes them from anxiolytics of the benzodiazepine series which, as we know, do not possess antiamnesic activity in therapeutic doses. Comparison of the substances with respect to different types of action shows that NMF and AZN, structural analogs of NAM, are much more active (by more than one order of magnitude of doses) than endogenous ligands of benzodiazepine receptors NAM and inosine, and that they are about equal in activity to diazepam. These results indicate that there are good grounds for making a search for new and effective psychotropic and nootropic drugs among compounds structurally similar to endogenous ligands of benzodiazepine receptors.

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