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Dissimilar Effects of Nicotinamide and Inosine, Putative Endogenous Ligands of the Benzodiazepine Receptors, on Pentylenetetrazol Seizures in Four Strains of Mice

IZYASLAW P. LAPIN

Laboratory of Psychopharmacology, Bekhterev Psychoneurological Research Institute, Leningrad, U.S.S.R.

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LAPIN, I. P. *Dissimilar effects of nicotinamide and inosine, putative endogenous ligands of the benzodiazepine receptors, on pentylenetetrazol seizures in four strains of mice.* PHARMAC. BIOCHEM. BEHAV. 13(3) 337–341, 1980.—In adult male albino BALB/c mice inosine (INS, 100 and 200 μg, intraventricularly) prolonged the latency of pentylenetetrazol (PTZ) seizures while nicotinamide (NAM) exerted an opposite effect. In adult male C57BL/6 mice INS decreased lethality after PTZ while NAM increased it. In adult male albino SHR (bred from Swiss) and in adult male CC57BR mice INS and NAM did not modify the effect of PTZ. Both INS and NAM administered ICV induced short-lasting locomotor excitement in albino SHR and BALB/c mice but not in C57BL/6 or CC57BR mice. Pretreatment with INS (300 mg/kg, IP) prolonged the latency of PTZ seizures only in SHR mice. Pretreatment with NAM was ineffective in all strains tested. Chronic treatment with NAM and INS (100 mg/kg, IP, daily for 5 days) in SHR mice did not modify the effect of PTZ. The data obtained emphasize the importance of the appropriate choice of mouse strain for studies on INS and NAM as putative endogenous ligands of the BDZ receptor (BDZR). The opposite effects of INS and NAM raise doubts that these two substances could play the same or similar roles in the function of a type of BDZR which is related to the action of PTZ on the central nervous system.

Inosine Nicotinamide Pentylenetetrazol Benzodiazepine receptor

THE present study was inspired by a paper [9] presented at the Symposium "Crucial Points in Psychiatric Research" in Moscow, May 23, 1979. In this paper it was suggested that nicotinamide (NAM) may be a putative endogenous ligand of the benzodiazepine receptor (BDZR) of the central nervous system. The authors based their conclusion on the pharmacological comparison in mice of NAM with two other putative endogenous ligands of the BDZR, inosine (INS) and hypoxanthine (HX), which appeared to be inactive. Data on these latter two compounds seemed inconsistent with a paper [14] identifiying INX and HX as endogenous inhibitors of ^{3}H-diazepam binding in the CNS. NAM has attracted our interest because during the last decade the metabolites of tryptophan of the kynurenine pathway (kynurenines) have been pharmacologically studied in our laboratory [5,6]. Unfortunately, NAM has not been studied as systematically as the other kynurenines. The convulsant effect of kynurenines injected into brain ventricles (ICV) in mice has been observed [6]. NAM, like anthranilic and xanthurenic acids, differed from other kynurenines by its lack of this convulsant effect [7]. Soon afterwards we extended our studies on NAM to find out whether or not it interacts with pentylenetetrazol (PTZ) and/or has other pharmacological effects which can be related to its activity as an endogenous ligand of the BDZR. Other kynurenines, including nicotinic acid, did not interact with PTZ in mice (this journal, in press). The data cumulated in our studies did not support the hypothesis of Möhler *et al.* [9] that NAM is an endogenous ligand of the BDZR possessing BDZ-like pharmacological activity [7]. In adult male albino SHR mice NAM (5–50 μg, ICV, or 25–250 mg/kg, IP) did not modify PTZ, strychnine, or thiosemicarbazide seizures. NAM did not alter the antiPTZ action of diazepam (0.5 μg, ICV or 0.1–0.5 mg/kg, IP), GABA (50 and 100 μg, ICV), or their combinations at subthreshold or effective doses. NAM changed neither sedative nor muscle relaxant effects of diazepam (1 mg/kg, IP or 5 μg, ICV) or GABA (50 μg, ICV). GABA has been applied in these studies because of numerous evidence of its involvement in the function of the BDZR [2]. We did not confirm an observation [9] of BDZ-like pharmacological effects of high doses (100–250 mg/kg, IP) of NAM [7]. We ran INS in identical tests and again, results were negative. INS did not interact with PTZ or diazepam. This observation disagrees with others [10, 15, 16] who have reported antiPTZ effects of INS in mice. One possible reason for the disagreements mentioned above could be the variations in the mouse strains used in these

TABLE 1

EFFECT OF NICOTINAMIDE AND INOSINE ADMINISTERED INTRACEREBROVENTRICULARLY ON PENTYLENETETRAZOL SEIZURES IN FOUR STRAINS OF MICE

Pretreatment	Dose	SHR				BALB/c				C57Bl/6				CC57Br			
		Effect of pentylenetetrazol (80 mg/kg IP)															
ICV	mcg	Latency min $\bar{n}\pm SE$	Number of mice Total	CS	L	Latency min $\bar{n}\pm SE$	Number of mice Total	CS	L	Latency min $\bar{n}\pm SE$	Number of mice Total	CS	L	Latency min $\bar{n}\pm SE$	Number of mice Total	CS	L
Saline	—	4.9±0.8	8	8	5	5.6±0.9	8	5	1	3.6±0.3	8	8	2	6.4±0.4	8	7	5
Nicotinamide	25	5.5±0.4	8	8	5	8.6±2.0	8	8	2	3.6±0.4	8	8	3	7.7±0.2	8	7	4
	50	3.7±0.2	7	6	6	3.5±0.2*	8	8	5	4.0±0.2	8	8	7†	7.4±0.3	8	7	6
	100	4.0±0.3	8	7	5	2.4±0.2‡	8	8	4	—	—	—	—	—	—	—	—
Saline	—	4.3±0.7	12	11	9	4.0±0.7	8	7	3	4.7±0.5	8	8	8	7.5±0.3	11	9	6
Inosine	50	7.6±1.7	12	11	5	—	—	—	—	3.1±0.3*	8	8	4	5.1±0.4*	10	8	4
	100	4.8±1.0	12	11	3	10.4±2.0‡	8	8	2	5.2±0.6	8	8	2†	6.8±0.5	12	11	4
	200	4.3±0.8	12	12	9	10.1±3.1	8	7	2	3.7±0.5	8	8	4	5.6±0.6	10	9	1*

Abbreviations: CS—clonic seizures, L—lethality,—not tested.
p (vs. control): *$p<0.05$; †$p<0.01$;‡$p<0.001$.

studies. We do not know the strain used in the experiments with NAM [9] because this was not mentioned in the paper or in the abstract. AntiPTZ effects of INS have been demonstrated [10, 15, 16] in C3H/HeN and NIH General Purpose strains. To investigate the role of strain differences we undertook the present study. In order to compare our data more precisely with those reported earlier, we added a series with chronic administration of NAM in one of the strains (see Method).

METHOD

Animals

The adult male mice were supplied by the Rappolovo farm near Leningrad. They were of about the same age (2.5–3 months), weighing 18–24 g (SHR—bred from Swiss), 18–21 g (BALB/c), 16–18 g (C57Bl/6) and 20–26 g (CC57BR). Mice were housed in a vivarium in metal boxes 30×80×20 cm in groups of 40 animals. They received the same food (cow milk, corn, and vegetables).

Drugs

Drugs were freshly dissolved in distilled water and injected intraperitoneally (IP) in a volume of 1 percent of body weight. Controls received the same volume of distilled water. Injection of drugs into brain ventricles (ICV) in conscious mice was made by a semiautomatic apparatus [17] according to the procedure described elsewhere [6]. The only difference was the use of distilled water instead of the methylene blue which had been administered before to verify the accuracy of injection in each mouse. Because this dose of methylene blue (about 10 μg) appeared to possess a slight inhibitory action, we did not use it in this study. Control of accuracy of ICV injections was made in each experiment in a separate "training" group of 5–8 mice of each strain using, as previously, a 0.25% solution of methylene blue. Accuracy of injection varied between 80 and 100 percent. Speed of ICV injection was about 10 sec per mouse. Water solutions of NAM and INS had pH's of 6.5 and 7.0, respectively.

Doses of NAM and INS ICV higher than 100 and 200 μg, respectively, were not used because motor excitement, and increased excitability were observed. A dose of 300 mg/kg IP of both compounds was used based upon the effective dose of NAM as a benzodiazepine-like drug reported elsewhere [9], and in accord with the pharmacologically effective doses of other metabolites of tryptophan in the mouse.

Chronic administration of NAM and INS was prompted by a report, published only in Russian, [9] that "NAM very poorly penetrates into the brain and therefore its specific activity might be demonstrated only after repeated injections". Although pharmacological benzodiazepine-like effects from single doses of NAM in mice have been shown in our work, a chronic administration of NAM and INS was used in the present study in order to provide optimal circumstances for the observation of benzodiazepine-like (or opposite) effects of NAM and INS.

Procedure

Pretreatment with NAM or INS ICV was made 1 min prior to PTZ and IP 30 min prior to PTZ. After injection of PTZ mice were kept in groups of 4–6 animals in metal boxes of 20×15×10 cm. In the series of chronic administration of NAM and INS IP, injections were made 1 hr before PTZ. Locomotion and rearings were measured in a single mouse according to the procedure described elsewhere [6]. Five criteria of the convulsant action of PTZ were registered: latency of clonic seizures, rates of clonic seizures and tonic extension per group, lethality and survival time. The latter did not differ between various groups. Observation lasted 40 min. Comparisons of the action of NAM (or INS) under the same route of administration in four strains were carried out in one day 11 a.m.–2 p.m. In SHR mice experiments were extended over several days. Some doses of NAM and INS were tested repeatedly on separate days. The present study was carried out December 1979–February 1980. Room temperature was 20–21°C.

Statistical Treatment of Data

Differences between groups in the latency and the survival time were analysed by the Student's t test. The rates of

TABLE 2

EFFECT OF SINGLE INTRAPERITONEAL ADMINISTRATION OF NICOTINAMIDE AND INOSINE ON PENTYLENETETRAZOL SEIZURES IN FOUR STRAINS OF MICE

Pretreatment	Dose	SHR				BALB/c				C57Bl/6				CC57Br			
		Effect of pentylenetetrazol (80 mg/kg, IP)															
IP	mg/kg	Latency min $\bar{n}\pm SE$	Number of mice Total	CS	L	Latency min $\bar{n}\pm SE$	Number of mice Total	CS	L	Latency min $\bar{n}\pm SE$	Number of mice Total	CS	L	Latency min $\bar{n}\pm SE$	Number of mice Total	CS	L
Dist. water	—	1.9±0.3	10	9	5	2.3±0.5	7	6	2	3.4±0.5	8	8	1	2.4±0.3	7	7	4
Nicotinamide	300	2.6±0.3	9	9	8	6.2±1.7	8	8	1	5.5±0.7	8	8	2	2.5±0.4	6	6	5
Inosine	300	3.4±0.7*	9	9	5	4.1±0.7	8	6	1	3.8±0.3	8	8	3	3.0±0.2	6	6	4

Abbreviations: see Table 1.

TABLE 3

EFFECT OF CHRONIC ADMINISTRATION OF NICOTINAMIDE AND INOSINE ON PENTYLENETETRAZOL SEIZURES IN SHR MICE

Groups	Pretreatment, IP		Effects of pentylenetetrazol (80 mg/kg, IP)				
n=10	Four days	Fifth day	Number of mice CS	TE	L	Latency min $\bar{n}\pm SE$	Survival time min $\bar{n}\pm SE$
1	Dist. water	Dist. water	9	4	4	1.9±0.8	5.3±2.3
2	Dist. water	Nicotinamide	9	6	6	1.5±0.3	8.0±2.1
3	Dist. water	Inosine	8	4	3	3.6±1.3	7.0±3.6
4	Nicotinamide	Nicotinamide	9	8	7	3.8±1.3	9.8±2.0
5	Inosine	Inosine	10	5	5	4.8±1.0*	10.2±3.0

Abbreviations—see Table 1.
Nicotinamide and inosine in a dose of 100 mg/kg once a day.
*$p<0.05$ (vs. group 1).

clonic seizures, tonic extension and lethality were compared by chi-square test.

RESULTS

Intracerebroventricular Administration of NAM and INS

The dose of PTZ (80 mg/kg) appeared to be equipotent in all strains. NAM was effective in only two strains: reducing the latency in BALB/c and increasing lethality in C57Bl/6 (Table 1). The effect on the latency seems to be dose dependent. INS prolonged the latency to PTZ seizures in BALB/c in doses of 100 and 200 μg. At the lowest dose tested (50 μg), it shortened the latency to PTZ seizures in C57Bl/6 and CC57BR (Table 1). INS decreased lethality in these two latter strains. There were no differences in either rate of tonic extension or survival time between controls and treated groups. Thus, NAM and INS exhibit opposite effects in the same strains, i.e., on the latency in BALB/c and on lethality in C57Bl/6. Mice of the SHR strain differed from the other strains in that they did not display the effects of NAM and INS mentioned above.

Strain differences were observed after ICV administration of NAM or INS without PTZ. In albino mice, SHR and BALB/c, both compounds produced locomotor excitement and an increase of reflectory excitability while in nonalbino strains, C57Bl/6 and CC57BR, there were no visible changes in behavior.

Single Intraperitoneal Administration of NAM and INS

A single dose of 300 mg/kg of both NAM and INS did not modify the effect of PTZ (Table 2). The only exception was an increase in the latency of seizures in SHR mice after INS. The reliability of this effect has not yet been tested. NAM and INS were also ineffective when they were administered in the same dose of 300 mg/kg 1 hr or 3 hr prior to PTZ in SHR mice. This dose of both compounds did not modify seizures induced by thiosemicarbazide (20 mg/kg, IP) or those induced by convulsant metabolites of tryptophan, l-kynurenine (50 μg, ICV) or quinolinic acid (5 μg, ICV). The only exception consisted of the shortening of the latency of seizures induced by quinolinic acid from 107.2±2.1 in controls to 77.0±5.2 ($p<0.001$) following INS. Single doses of NAM and INS of 100 and 250 mg/kg were ineffective in PTZ treated SHR mice. In this strain hypoxanthine (250 mg/kg, IP) was ineffective as well.

Chronic Intraperitoneal Administration of NAM and INS

Administration of NAM and INS over 5 days did not result in any change in the response to PTZ (Table 3). After chronic INS administration the latency of seizures was

longer than after chronic administration of a vehicle. However the difference between groups treated with INS chronically or acutely (one injection day) was not significant. All five groups of SHR mice (Table 3) did not differ from each other in initial body weight and motor activity (locomotion and rearings) on the 1st day of the experiment or on the 5th day of the experiment immediately before injection of PTZ.

DISCUSSION

The antiPTZ effect of INS administered ICV was observed in the present study in BALB/c mice (prolongation of the latency) and in C57BL/6 mice (decrease of lethality). Although the effect of INS on the latency was not dose dependent, and in this respect differs from that described earlier [10, 15, 16], the efficacy of INS in BALB/c mice suggests that this strain may be similar to C3H/HeN and NIH General Purpose strains in their susceptibility to the antiPTZ effect of INS. When one compares these strains it is noteworthy that mice of the latter two strains had been lightly anesthetized with diethyl ether and an incision had been made in the scalp to expose bregma whereas mice of all four strains in the present study were conscious. Other details of the method were very similar, e.g., ICV route of administration, doses, time intervals, etc. Thus, BALB/c strain seems to be the most suitable of the four strains tested for studying INS as an endogenous BDZ-like compound.

The BALB/c strain has been described as being different from other mouse strains in that there is lower binding of ^{3}H-diazepam with the BDZR and a lower density of the BDZR in the brain [11]. This strain is less susceptible to the inhibitory action of BDZs. This has been demonstrated by the facilitation of avoidance training with no avoidance inhibition at higher doses of chlordiazepoxide [12] and by the stimulant action of low doses of phenazepam which in other strains produced only sedation [13]. The antiPTZ effect of INS which was manifested in prolongation of the latency and in a decrease of lethality appeared to be rather moderate, and it is many times weaker than that of BDZs which completely prevent seizures at much lower doses. Moreover this effect of INS was not observed in some mouse strains in the present study as well as in rats (male Charles River) after intracisternal injection [1]. It seems to be important that the effect of an *endogenous* compound on brain receptors has to be more or less universal, at least in mammals, in order to accept its physiological significance. Because this is not the case we have to share in a conclusion [3] that the physiological or pharmacological significance of an interaction of some purines with the BDZR remains questionable.

As for NAM, our data are opposite to those reported elsewhere [9]. NAM did not exert any BDZ-like pharmacological action after either single ICV or IP injections or chronic IP administration (Tables 1–3). On the contrary, it had effects opposite to those of INS in the same susceptible mouse strains, i.e., BALB/c and C57BL/6. NAM dose-dependently shortened the latency of PTZ seizures and increased lethality. These effects, which are opposite to expected BDZ-like ones, do not disclaim an interaction of NAM with the BDZR. It seems reasonable that "if INS or HX interact with the BDZR in vivo, it is likely they will either mimic or antagonize the pharmacologic effects of the BDZs" [15] or, let us add, possess action opposite to that of BDZs. The opposite effects of INS and NAM observed in the present study raise an objection against the suggestion that both compounds act identically on the same type of BDZR. It is likely that NAM exerts either PTZ-like action on the BDZR which mediates the action of PTZ, or an unknown action on another BDZR which then sensitizes the former BDZR towards PTZ. Recently two biochemically and pharmacologically distinct BDZRs [4] and new endogenous "BDZ-like" compounds from brain [8] have been described. One may suspect that the antiPTZ effect of BDZs is more related to the type I BDZR which is thought [4] to mediate the anxiolytic actions of BDZs. This suspicion is based on the high correlation between antiPTZ and antianxiety effects of BDZ, including the strongest anxiolytic BDZ, phenazepam [18], which has an ED_{50} in the antiPTZ test of 0.037 mg/kg vs 0.51 mg/kg for diazepam and 4.6 mg/kg for chlordiazepoxide. It is possible to speculate that NAM could act, at least in BALB/c and C57BL/6 mice, on the type II BDZR which mediate [4] pharmacological effects other than anxiolytic activity.

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