Pharmacology Biochemistry & Behavior, Vol. 14, pp. 589–593, 1981. Printed in the U.S.A.

Nicotinamide, Inosine and Hypoxanthine, Putative Endogenous Ligands of the Benzodiazepine Receptor, Opposite to Diazepam Are Much More Effective Against Kynurenine-Induced Seizures Than Against Pentylenetetrazol-Induced Seizures

IZYASLAV P. LAPIN

Laboratory of Psychopharmacology, Bekhterev Psychoneurological Research Institute, Leningrad, USSR

Received 9 December 1980

LAPIN, I. P. *Nicotinamide, inosine and hypoxanthine, putative endogenous ligands of the benzodiazepine receptor, opposite to diazepam are much more effective against kynurenine-induced seizures than against pentylenetetrazol-induced seizures.* PHARMAC. BIOCHEM. BEHAV. 14(5) 589–593, 1981.—Nicotinamide (NAM, 1000 mg/kg), inosine (INS, 1000 mg/kg), hypoxanthine (HXT, 500 mg/kg), putative endogenous ligands of the benzodiazepine receptor, and nicotinic acid (NA, 500 mg/kg) diminished DL-kynurenine-(DL-K, 50 μg ICV) induced seizures in C57BL/6 adult male mice and only prolonged the latency of pentylenetetrazol (PTZ, 500 μg ICV) seizures. The same effect was previously observed when PTZ was administered IP. In albino male BALB/c and SHR (bred from Swiss) mice only NA was effective against DL-K. Diazepam in a dose of 0.5 mg/kg prevented PTZ-induced seizures in half of the animals but even in dose of 10 and 20 mg/kg it was ineffective against DL-K. When injected ICV NAM (1 and 10 μg), INS (10 μg) and HXT (10 μg) prevented seizures induced by DL-K and were ineffective against seizures induced by PTZ. It is suggested that if NAM, INS and HXT are of functional importance in the central nervous system, they can act as antagonists of endogenous brain kynurenine. NA and NAM are suggested to be functional feedback inhibitory regulators of the kynurenine pathway of metabolism of tryptophan.

Nicotinamide Inosine Hypoxanthine Kynurenine Pentylenetetrazol

HIGH doses (500–2000 mg/kg) of the putative endogenous ligands of the benzodiazepine receptor (BDZR), i.e., nicotinamide (NAM), inosine (INS), hypoxanthine (HXT), and of nonligand nicotinic acid (NA) antagonize pentylenetetrazol (PTZ) seizures in mice of various strains and this antagonism is evident only in prolongation of the latency of seizures (in press). This effect is not selective because ligands similarly antagonized other convulsants, e.g., strychnine and DL-kynurenine sulfate (DL-K). It is generally known (see [8]) that diazepam, a standard benzodiazepine (BDZ) tranquilizer, highly selectively antagonizes PTZ. Intracerebroventricularly (ICV) administered ligands in doses of 50–300 μg also prolonged the latency of PTZ seizures in BALB/c [7] and C57BL/6 mice (in press).

The purpose of the present study was to compare more precisely susceptibility of DL-K-induced and PTZ-induced seizures towards ligands or, in other words, comparative efficacy of ligands against DL-K and PTZ. It seemed reasonable that NAM and NA as tryptophan metabolites in the kynurenine pathway (kynurenines) could relate to DL-K equally or greater than to exogenous PTZ.

METHOD

Animals

Adult male mice of three strains, C57BL/6, SHR (bred from Swiss) and BALC/c, weighing respectively 18–20, 17–19 and 21–23 g were supplied by the Rappolovo farm.

Procedure

Procedure followed has been recently described in detail [3–7]. Injection of drugs into brain ventricles (ICV) in conscious mice was made by semiautomatic apparatus. IP pretreatment was made 30 min and ICV pretreatment 10 min prior to a convulsant.

TABLE 1

COMPARISON OF EFFECTS OF INTRAPERITONEALLY INJECTED PUTATIVE ENDOGENOUS LIGANDS OF THE BENZODIAZEPINE RECEPTOR ON SEIZURES INDUCED BY DL-KYNURENINE AND PENTYLENETETRAZOL

Pretreatment		Convulsant ICV		Effect			
IP	Dose mg/kg		Dose μg	Latency to CC sec, mean ± S.E.	Number of mice Total CC	TE	L
C57BL/6 strain							
D.W.	—	DL-K	50	127.6 ± 16.1	20 15	5	5
NAM	1000			181.0 ± 59.7	20 5‡	2	2
INS	1000			267.0	19 2‡	0*	0*
HXT	500			118.7 ± 8.9	19 4‡	0*	0*
NA	500			180	10 1‡	0*	0*
D.W.	—	DL-K	50	91.4 ± 20.9	8 7	1	1
Diazepam	10			73.7 ± 9.7	8 8	0	0
	20			90.0 ± 10.5	8 5	0	0
D.W.	—	PTZ	500	50.0 ± 21.8	15 9	0	1
NAM	1000			286.0 ± 59.4‡	9 4	0	0
INS	1000			119.0 ± 44.0	10 5	3	2
HXT	500			146.2 ± 33.0*	10 5	0	0
SHR strain							
D.W.	—	DL-K	50	79.3 ± 8.7	10 8	3	3
NAM	1000			90.0 ± 5.3	10 6	2	2
INS	1000			123.3 ± 23.7	10 6	0	0
HXT	500			83.3 ± 6.2	10 9	4	4
NA	500			156.6 ± 31.8*	10 3*	0	0
D.W.	—	PTZ	500	93.4 ± 17.3	20 13	2	2
NAM	1000			152.4 ± 52.3	10 5	1	2
INS	1000			47.0 ± 15.8	10 6	3	3
HXT	500			108.0 ± 10.1	10 6	2	2
BALB/c strain							
D.W.	—	DL-K	50	65.0 ± 10.3	10 8	0	0
NAM	1000			66.8 ± 7.5	10 8	0	0
INS	1000			110.0 ± 17.9*	10 6	0	0
HXT	500			80.8 ± 10.6	10 6	0	0
NA	500			210.2 ± 3.9‡	10 2*	0	0

Abbreviations: D.W.—distilled water, NAM—nicotinamide, INS—inosine, HXT—hypoxanthine, NA—nicotinic acid, DL-K—DL-kynurenine sulfate, PTZ—pentylenetetrazol, CC—clonic convulsions, TE—tonic extension, L—lethality.

*$p<0.05$, †$p<0.02$, ‡$p<0.01$.

Drugs

NAM (Koch-Light), INS (Reanal), HXT (Reanal) and NA (city drug store) were dissolved in distilled water. Solutions of HXT required heating and were not available stronger than 1% thus limiting use of higher doses ICV. Solutions of all pretreatment drugs had pH 6–7. In some experiments 2.5% ampuled solutions of NAM (city drug store) dissolved by distilled water were used. Special comparison of preparations of NAM from two mentioned sources showed their equal activity. DL-kynurenine sulfate monohydrate (DL-K) and quinolinic acid (QA), both from Sigma Co., were also dissolved in distilled water. PTZ (city drug store) was used as 10% ampuled solution.

Statistical Treatment of Data

Differences between groups in the latency of seizures were treated with Student's t test and those in the rates of clonic seizures, tonic extension and lethality with Chi-square test.

RESULTS

IP administered NAM, INS, HXT and NA diminished rate of DL-K-induced clonic seizures per group and, except NAM, of tonic extension and lethality in C57BL/6 mice (Table 1). Diazepam appeared to be ineffective in doses of 10 and 20 mg/kg in C57BL/6 mice (Table 1) and SHR mice while in a dose of 0.5 mg/kg it prevented seizures and lethality in

TABLE 2

EFFECT OF PUTATIVE LIGANDS AND NICOTINIC ACID ON BODY TEMPERATURE AND MOTOR ACTIVITY (CONTROL)

Drug	Dose mg/kg	Hypothermia 30 min after IP injection −Δt*, mean ± S.E. C57BL/6	SHR	Motor activity Locomotion SHR	Rearings strains
D.W.	—	1.3 ± 0.2	1.6 ± 0.2	11.7 ± 1.9	8/8
NAM	1000	3.8 ± 0.3‡	5.0 ± 0.5‡	10.3 ± 0.8	2/8†
INS	1000	2.9 ± 0.4*	4.2 ± 0.6‡	9.2 ± 1.7	4/8
HXT	500	2.2 ± 0.5	2.8 ± 0.4	14.0 ± 2.0	8/8
NA	500	—	4.7 ± 0.1‡	3.4 ± 0.6†	1/8†

Abbreviations: see Table 1.
Locomotion—number of lines crossed.
Rearings—number of mice reared to total number of mice in group.

TABLE 3

COMPARISON OF EFFECTS OF INTRACEREBROVENTRICULARLY INJECTED PUTATIVE ENDOGENOUS LIGANDS OF THE BENZODIAZEPINE RECEPTOR ON SEIZURES INDUCED BY DL-KYNURENINE AND PENTYLENETETRAZOL

Pretreatment ICV	Dose μg	Convulsant ICV	Dose μg	Effect Latency to CC, sec mean±S.E.	Number of mice Total	CC	TE	L
SHR strain								
Saline	—	DL-K	50	58.0 ± 8.6	10	8	4	6
NAM	0.1			176.0 ± 23.3†	10	4	0*	0†
	1.0			112.0	10	2†	0*	0†
	10.0			57.5	10	2†	0	0†
Saline	—	DL-K	50	70.0 ± 2.4	10	7	2	1
INS	1.0			101.0 ± 16.4	10	4	0	0
	10.0			—	10	0‡	0	0
HXT	1.0			91.6 ± 38.8	10	4	0	0
	10.0			345	10	1‡	0	0
Saline	—	PTZ	500	36.6 ± 5.6	10	9	4	4
NAM	1.0			62.5 ± 12.9	10	8	5	5
	10.0			68.7 ± 17.4*	10	4	3	1
	100.0			18.5 ± 3.7	10	7	4	4
INS	10.0			34.2 ± 2.7	10	7	0	0
	100.0			30.0 ± 3.8	10	5	1	1
Saline	—	PTZ	500	43.0 ± 5.2	10	5	1	1
HXT	10.0			37.1 ± 2.4	10	7	1	1

Abbreviations: see Table 1.

half of the SHR and C57BL/6 mice treated with PTZ (80 mg/kg IP or 500 μg ICV). The same doses of NAM, INS and HXT did not diminish seizures but only prolonged the latency of seizures induced by PTZ administered ICV (Table 1). When PTZ was injected SC or IP, pretreatment with NAM, INS and HXT gave similar results (in press).

Putative ligands in doses used in C57BL/6 mice were practically not effective in albino SHR and BALB/c mice treated with DL-K (Table 1). NAM in a dose of 1000 mg/kg in some other experiments prolonged the latency of DL-K seizures. Ligands also did not modify PTZ seizures in SHR mice (BALB/c mice were not tested against PTZ administered ICV). However, NA increased the latency and diminished rate of clonic seizures induced by DL-K in these two albino strains (Table 1).

Control measurements have found (Table 2) that the doses of tested compounds used, except HXT, lowered body temperature and two of them, NAM and NA, inhibited rear-

ings. NA inhibited locomotion as well. Degree of hypothermia was slightly greater in SHR mice than in C57BL/6 mice.

The convulsant effect of another tryptophan metabolite with convulsive action, QA (5 μg, ICV), was not modified by the doses of ligands used with SHR and C57BL/6 mice. NA in a dose of 500 mg/kg diminished the number of C57BL/6 mice with clonic seizures and prolonged the latency of QA seizures in SHR mice.

ICV injection of NAM, INS and HXT reduced the number of SHR mice, a strain in which these compounds were ineffective under IP administration, with DL-K-induced clonic seizures (Table 3). These doses of compounds and a dose of 100 μg of NAM and INS practically did not change the convulsant action of PTZ administered ICV (Table 3) or IP (80 mg/kg). An exception was prolongation of the latency of PTZ seizures in mice treated with 10 μg of NAM. This dose of NAM, INS and HXT did not change body temperature and motor activity 10 min after ICV injection in control mice. Doses of HXT ICV higher than 10 μg were not tested because of poor solubility of this compound in water. NA at doses of 0.1 μg and 1.0 μg ICV prevented DL-K induced seizures in SHR mice.

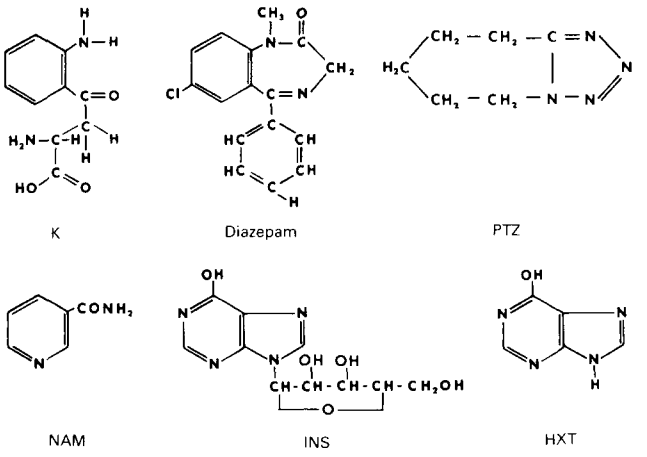

FIG. 1. Chemical structures of compounds studied.

DISCUSSION

Data obtained show that in C57BL/6 mice, a strain which is particularly susceptible to the anti-PTZ action of putative ligands (in press, see also [7], these compounds are much more effective against DL-K than against PTZ (Table 1). In albino BALB/c and SHR mice, ligands in the doses used were not effective against DL-K while NA was. The latter was the most effective against DL-K, also in C57BL/6 mice as in a previous study (in press) against PTZ. Observed differences between C57BL/6 and SHR strains in susceptibility to anti-DL-K action of tested compounds could be due to poorer penetration into brain in SHR mice since all compounds were highly effective in SHRs when injected ICV. We did not study so far the anti-DL-K action of ligands administered ICV in BALB/c mice.

Because it is NA which most markedly inhibited locomotion and rearings and lowered body temperature one may wonder whether anti-DL-K effect of NA and of other studied compounds is due to their nonspecific inhibitory action. Hypothermia is known [1] to decrease transport of amino acids, e.g., GABA, which counteracts DL-K (in press) in the brain, and it is likely that hypothermia is of importance for distribution of an amino acid metabolite, DL-K, from brain ventricles into brain structures. Moreover, hypothermia is of some importance for protection of SHR mice against audiogenic seizures [4]. Three facts speak against an idea that hypothermia can be a determinant of anti-DL-K effect of ligands and demonstrate that there is no correlation between lowering of temperature and protection against DL-K seizures: (1) In C57BL/6 mice HXT did not lower body temperature (Table 2) but was equipotent to NAM which appeared to be the most hypothermic among ligands. (2) In SHR mice NA and NAM equally lowered body temperature (Table 2) but only the former diminished DL-K seizures. (3) In SHR mice ICV administration of ligands in a protective dose of 10 μg did not lower body temperature. These arguments bring doubts that hypothermia is important for anti DL-K effect of NA as well.

It seems premature to speculate whether the anti-kynurenine effect of ligands and NA is central or peripheral or both. Because ligands in small doses ICV prevented DL-K seizures it seems reasonable that the anti-kynurenine effect of ligands, both endogenous and injected IP or ICV, is of central origin. Some peripheral mechanisms may also be involved in protection against convulsants as it has been shown [4] for IP administered kynurenines.

Much higher activity of ligands against DL-K than against PTZ suggests that NAM, INS and HXT can be considered as endogenous antagonists of endogenous brain kynurenine. Whether their action is related to occupation of the BDZR remains an open question.

Chemical structures of DL-K (and natural L-K; for both, further abbreviation K) and diazepam can be represented in a manner showing their close similarity (Fig. 1) which suggests that K can occupy if not the whole BDZR, a part (a supplement) of this receptor and, when so, K can be considered as a putative endogenous ligand of the BDZR.

There are three fragments (F), or moieties, common in the structures of K and diazepam.

F_1 N–C–C–C=R; R=O in K and R=N in diazepam;

F_2 N–C–C=O; this F_2 is situated inside the benzodiazepine ring in diazepam and in the side chain in K.

In the structure of K one CH_2 group separates F_1 and F_2, while in the structure of K they are not separated.

F_3 C–C–C–C=R; R=O in K and R=C in diazepam; F_3 forms the contour of the lower part of both molecules. F_1 has similar spheric contour at benzyl ring of both molecules.

It seems important that only these two molecules, K and diazepam, have *three* common fragments. NAM and diazepam have only *one* common F_1. Purines HXT and INS (as well as adenine and adenosine) and diazepam have *two* common Fs: F_2 (instead of =O they have −OH) and F_1 N–C–C–N=(which is similar to F_1 differing by N=instead of C= in F_1).

Stimulant and convulsant effects of K are probably related to the presence of two carboxy groups (C=O) separated by two carbon atoms (C–C), i.e. to a moiety O=C–C–C–C=O. This moiety of the molecule is also typical of QA, the strongest endogenous convulsant among kynurenines, and aspartic acid, an excitatory amino acid. In the molecule of caffeine there are also two carboxy groups separated by one N atom. Similarly to K and diazepam caf-

feine has F_2 and modified F_1 (N–C–N–C=O) which suggest a binding of caffeine with the BDZR.

Inefficacy of diazepam against K might be due either to replacement of diazepam by K as a more strongly competitive antagonist or to independence of seizures induced by K on its binding with BDZR. However, antagonism between the endogenous ligands of the BDZR (although possessing low affinity), NAM, INS, HXT, and K speaks against the latter suggestion. It appears noteworthy that although chemical structures of ligands are much more similar to the structure of PTZ than to the structure of K (Fig. 1) they antagonize the latter much more effectively than the former. This could be because PTZ is a stronger competitor than ligands or because K and ligands bind with one part of the BDZR while PTZ binds with another part of this receptor. The above considerations require in vitro studies on replacement of ^{3}H-diazepam by K.

Anti-DL-K effect of NAM and NA, end-products of the kynurenine pathway of metabolism of tryptophan, deserves special attention. It has been recently shown [2] that not one of IP injected kynurenines in rats inhibits liver tryptophan pyrrolase activity. That discriminates this enzyme from the majority of other enzymes which are regulated by the inhibitory action of their products. In view of this fact a *functional* regulation of this pathway by a feedback inhibition of neuroactivity of kynurenine by its metabolites seems to be of particular importance for regulation.

REFERENCES

1. Banay-Schwartz, M., K. Lajtha, H. Sershen and A. Lajtha. Temperature dependence of amino acid transport in brain slices. *Neurochem. Res.* **2:** 695–706, 1977.
2. Kiseleva, I. P., I. P. Lapin, I. B. Prakhie and M. L. Samsonova. Dissimilar effects of kynurenines on rat liver tryptophan pyrrolase activity. *Vop. med. Khim.* **26:** 458–461, 1980.
3. Lapin, I. P. Stimulant and convulsive effects of kynurenines injected into brain ventricles in mice. *J. Neural Trans.* **42:** 37–43, 1978.
4. Lapin, I. P. Kynurenines and audiogenic excitement in mice. *Pharmac. Biochem. Behav.* **13:** 9–15, 1980.
5. Lapin, I. P. Effect of kynurenine and quinolinic acid on the action of convulsants in mice. *Pharmac. Biochem. Behav.* **13:** 17–20, 1980.
6. Lapin, I. P. Taurine selectively antagonizes L-kynurenine produced seizures in mice. *J. Neural Trans.* **48:** 311–316, 1980.
7. 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:** 337–342, 1980.
8. Vikhlayev, Yu. I. and T. A. Voronina. The pharmacological activity spectrum of phenazepam. In: *Minor Tranquillizers in Treatment and Rehabilitation of Psychoneurological and Psychosomatic Patients*. Leningrad: Bekhterev Institute, 1979, pp. 5–14.