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Short communication

ANTAGONISM OF L-GLYCINE TO SEIZURES INDUCED BY L-KYNURENINE, QUINOLINIC ACID AND STRYCHNINE IN MICE

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L-glycine (1-12.5 μg, intracerebroventricularly, i.c.v.) completely prevented seizures induced by i.c.v. administration of L-kynurenine, and practically did not modify those induced by another convulsant quinolinic acid, a metabolite of tryptophan, and by strychnine. L-Glycine administered intraperitoneally (i.p.) (1000 mg/kg) decreased lethality after K-kynurenine and quinolinic acid; at doses of 3000 and 4000 mg/kg which are sedative and hypothermic it prolonged the latency of strychnine and L-kynurenine seizures. The convulsant action of pentylenetetrazol was not modified. Kynurenine seizures are suggested to be related to the action of kynurenine on glycine receptors in the central nervous system.

Glycine Kynurenine Quinolinic acid Strychnine Seizures

1. Introduction

Experimental studies on metabolites of tryptophan from the kynurenine pathway (kynurenines) as putative endogenous compounds involved in the genesis of epilepsy (Lapin, 1978a,b; 1979; Gusel and Mikhailov, 1979) led to a comparison of kynurenines with typical convulsants and to an investigation of the interaction of these two groups of compounds. It has been observed that the most active convulsant kynurenines, L-kynurenine (L-K) and quinolinic acid (QA), potentiate seizures induced by strychnine (S) but not by pentylenetetrazol, thiosemicarbazide or sound (in press). Both kynurenines potentiate the in vitro inhibition of uptake of serotonin in human blood platelets by convulsants, particularly by S (in press). Enzymes metabolising γ-aminobutyric acid (GABA) were inhibited in the brain by L-K and QA and this suggested that these substances may diminish GABA-mediated inhibitory processes in the brain (Lapin et al., 1979). Together the above data suggested (Lapin et al., 1979) that kynurenines as convulsant agents can be classed according to the probable mechanism of their action with a group of drugs which inhibit inhibitory brain systems, e.g. S, picrotoxin, bicucullin, penicillin (Woodbury and Kemp, 1977). Because S appeared to be the most similar in action to L-K and QA, we turned our attention to the glycine (G) receptors (Snyder et al., 1973; Snyder, 1975) and to antagonism of S to G (Young and Snyder, 1973) as background for a hypothesis of the mechanism of action of S. The purpose of the present study was to find out whether G modified seizures induced by L-K and QA and to relate this to its effect on the convulsant action of S.

2. Materials and methods

Albino male mice (bred from Swiss) weighing 18—22 g from Rappolovo farm were used. Aqueous solutions of S sulfate and L-G PFA

(analytical grade were injected s.c. or i.p., resp., in a volume of 0.01 ml/g. Injections of aqueous solutions of S, L-G, L-K sulfate and QA (two latter from Sigma Co.) into the brain ventricles (i.c.v.) of conscious mice were made by means of a semiautomatic apparatus (Vaneček et al., 1960) in a volume of 0.005 ml. Controls were treated with saline. The procedure for i.c.v. injection has been described in detail elsewhere (Lapin, 1978a). The only difference was that methylene blue was not used as a vehicle (to control accuracy of injection in each mouse) because the dose of about 10 μg appeared not to be pharmacologically indifferent. The accuracy of i.c.v. injections was controlled at the beginning of each experiment in a group of 5—6 mice injected with methylene blue. The accuracy of injections in these tests varied from 80 to 95%. Exploratory locomotion and rearings were measured in individual mice for 2 min by methods described elsewhere (Lapin, 1978a) 30 min after i.p. injection of L-G. Pretreatment with L-G was made 30 min (i.p.) of 5 min (i.c.v.) prior to other drugs. After treatment with S, L-K and QA the mice were placed in metal boxes (20 × 15 × 10 cm) in groups of 4. Room temperature was 19—21°C. The mice were observed for 30 min after injection of S, L-K and QA. Rectal temperature was measured by an electrothermometer. The statistical significance of differences was calculated according to χ^2 method and to Student's *t*-test.

3. Results

Pretreatment with L-G (i.p.) slightly prolonged the latency of seizures induced by S, but did not diminish either seizures or lethality. At doses of 1000 and 2000 mg/kg, L-G prolonged the latency in some experiments but this effect was not properly reproducible. Effective doses of L-G were 3000 and 4000 mg/kg (table 1). These doses were sedative and hypothermic. In a dose of 6000 mg/kg L-G exhibited a toxic action i.e. produced discoordination and ataxia. This dose did not modify the action of S.

TABLE 1

Effect of L-glycine on the latency of strychnine seizures. Groups of 10-12 mice were used.

Pretreatment with L-glycine	Latency min $\bar{m} \pm$ S.E.
i.p., mg/kg	*Treatment with strychnine 1.5 mg/kg, s.c.*
Distilled water	11.6 ± 0.5
3000	18.2 ± 1.5 [c]
4000	24.1 ± 1.7 [c]
i.c.v., μg	
Saline	11.8 ± 0.7
1.0	13.2 ± 3.0
5.0	14.5 ± 2.8
50.0	7.6 ± 1.1 [b]
100.0	5.0 ± 1.0 [b]
	10 μg, i.c.v.
Saline	0.7 ± 0.1
5.0	2.1 ± 0.2 [c]

[a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$; [d] $P < 0.001$.

At doses of 50—1600 mg/kg, L-G did not change locomotion and rearings. In a dose of 2000 mg/kg it inhibited rearings (from 13.5 ± 3.3 in controls to 5.5 ± 1.7) and in a dose of 3000 mg/kg it inhibited both rearings (to 1.2 ± 0.4) and locomotion (from 19.5 ± 1.7 in controls to 11.5 ± 2.3).

In a dose of 1000 mg/kg L-G diminished the lethality in mice treated with L-K and QA and the tonic extension induced by the latter (table 2). This dose of L-G did not lower rectal temperature. The convulsive action of pentylenetetrazol (80 and 90 mg/kg i.p. or s.c.) was not modified by pretreatment with L-G (5 μg, i.c.v. or 1000 and 2000 mg/kg, i.p.). The latency of thiosemicarbazide (20 mg/kg, s.c.) seizures was prolonged by L-G (2000 and 3000 mg/kg, i.p.) administered 30 min after thiosemicarbazide. At doses of 3000 and 4000 mg/kg, L-G prolonged the latency and decreased lethality after L-K (100 μg, i.c.v.). The rate of seizures was diminished only by the highest dose of L-G used (4000 mg/kg).

TABLE 2

Effect of L-glycine on seizures induced by L-kynurenine (L-K) and quinolinic acid (QA). Statistical significance of differences ses table 1.

Pretreatment with L-glycine	Treatment with kynurenines i.c.v., μg		Number of mice				Latency of clonic seizures min $\bar{m} \pm$ S.E.
			Total	Clonic seizures	Tonic extention	Lethality	
i.p., mg/kg							
Distilled water	L-K	100	12	10	1	10	1.5 ± 0.2
1000	L-K	100	12	10	0	4 [b]	1.1 ± 0.2
Distilled water	L-K	100	12	12	1	9	1.0 ± 0.1
3000	L-K	100	8	7	0	2 [a]	1.9 ± 0.1 [b]
4000	L-K	100	12	5 [a]	0	0 [c]	3.3 ± 0.3 [b]
Distilled water	QA	10	12	8	8	8	1.8 ± 0.3
1000	QA	10	12	5	1 [b]	3 [b]	3.4 ± 0.5
i.c.v., μg							
Saline	L-K	50	12	10	3	5	1.6 ± 0.1
0.2	L-K	50	12	4 [a]	0	2	4.5 ± 1.6 [a]
Saline	L-K	50	12	7	4	3	2.0 ± 0.4
1.0	L-K	50	12	0 [c]	0	0	—
5.0	L-K	50	12	0 [c]	0	0	—
Saline	L-K	50	12	7	4	3	1.8 ± 0.1
12.5	L-K	50	12	0 [c]	0	0	—
Saline	QA	5	16	8	7	7	4.1 ± 0.3
1.0	QA	5	12	9	7	3	1.8 ± 0.2 [a]
5.0	QA	5	10	6	6	4	3.0 ± 0.4
12.5	QA	5	12	9	5	5	2.5 ± 0.4

When injected i.c.v. L-G (1-12.5 μg) prevented the seizures and lethality induced by L-K while the effects of QA were not diminished. The latency of clonic seizures after i.c.v. administration of S was slightly increased (table 1) and that after QA was decreased (table 2). With the i.c.v. route of administration, L-G in doses of 1—25 μg did not modify either the latency or the rate of seizures and the lethality after s.c. treatment with S (1.5 mg/kg). The threshold protective dose of L-G against L-K was 0.2 μg.

At doses of 5 and 10 μg L-G, also prevented the stimulation of locomotion induced by L-K (10 μg).

Higher doses of L-G (50 and 100 μg) shortened the latency of seizures induced by S. However these doses of L-G already had a stimulant action. A dose of 200 μg of L-G appeared to be convulsant (CD_{75}) and lethal (LD_{50}).

4. Discussion

A strong protective effect of L-G against seizures induced by L-K seemed to be the most important finding in the present study. Because it was observed when both drugs were injected into brain ventricles one may suggest that their interaction appeared at structures situated adjacent to ventricles. Control tests showed that there was no inactivation of L-K by L-G in vitro or in brain ventricles when a mixture of the two drugs was administered. Presumably the hippocampus is one of those structures. Microinjection of L-K into the hippocampus increased the penicillin-induced epileptiform activity of the focus in this structure in frogs (Gusel and Mikhailov, 1979). The convulsant and lethal effects of QA, like those of S, were antagonized by L-G on i.p. but not i.c.v. administration. This observation discriminates between the convul-

sant actions of L-K and QA which so far have been reported as qualitatively similar (Lapin, 1978a,b; Lapin et al., 1979; Gusel and Mikhailov, 1979) and differing only quantitatively: QA was about 10 times (according to the effective doses in μg) or 20 times (according to molecular weights stronger. Shortening of the latency of QA seizures in mice pretreated with 1.0 μg of L-G (table 2) was a reproducible effect.

The fact that i.p. L-G decreased the lethality and rate of clonic seizures and prolonged the latency in mice treated with L-K (table 2) while even at the highest doses tested (3000 and 4000 mg/kg) it merely prolonged the latency of strychnine seizures (table 1) suggests that the antagonism of L-G against L-K was much stronger than that against strychnine. Similarity between interactions of L-G with QA and S suggests that they occur at the level of the spinal cord and to a lesser degree in the brain. In this respect it is noteworthy that QA has been described as qualitatively similar to L-K when they have been compared by using microinjections into frog hippocampus (Gusel and Mikhailov, 1979). Comparison of molecular weights of the drugs (S = 436, L-G = 75, L-K = 324, QA = 167) does not devaluate the suggestion of selective antagonism between L-G and L-K.

The same conclusion about the efficacy of L-G will have to be reached on the basis of the molar ratios of the doses. Prolongation of the latency of thiosemicarbazide seizures in mice pretreated with L-G could have been partially due to the hypothermia produced by high doses of L-G (2000 and 3000 mg/kg) and partially to the inhibitory action of L-G which presumably 'compensate' the decrease of brain GABA level produced by thiosemicarbazide. It seems probable that the effect of L-K and QA (and presumably of some other kynurenines with a convulsant action) on brain glycine receptors may be even more important for their convulsant action than is their interaction with GABA system. The strong and selective antagonism of L-G to L-K suggests a high affinity of glycine receptors for L-K.

References

Gusel, V.A. and I.B. Mikhailov, 1979, Increase of the epileptogenic focus activity in the frog hippocampus by kynurenines and its reduction by serotonin, Bull. Exp. Biol. Med. (Moscow) 87, 158.

Lapin, I.P., 1978a, Stimulant and convulsive effects of kynurenines injected into brain ventricles in mice, J. Neural Transm. 42, 37.

Lapin, I.P., 1978b, Convulsions and tremor in immature rats after intraperitoneal injection of kynurenine and its metabolites, Pharmacol. Res. Commun. 10, 81.

Lapin, I.P., 1979, Experimental studies on kynurenines as neurotropic tryptophan metabolites: past (1970—76), present (1977—79), future (1980—?), Trends Pharmacol. Sci. (in press).

Lapin, I.P., Z.S. Nikitina and I.A. Sytinsky, 1979, Inhibition of activity of enzymes of GABA metabolism by convulsant kynurenines, Vopr. Med. Khim. (Moscow) (in press).

Snyder, S.H., 1975, The glycine synaptic receptor in the mammalian central nervous system, Br. J. Pharmacol. 53, 473.

Snyder, S.H., A.B. Young, J.P. Bennett and A.H. Mulder, 1973, Synaptic biochemistry of amino-acids, Fed. Proc. 32, 2039.

Vaneček, J., V. Krebs, E. Scheer and T. Bieleke, 1960, A note on an apparatus for intracerebral injections in conscious mice, J. Am. Pharm. Assoc. 49, 178.

Woodbury, D.M. and J.W. Kemp, 1977, Basic mechanisms of seizures: neurophysiological and biochemical etiology, in: Psychopathology and Brain Disfunctions, eds. C. Shagass, S. Gershon and A.J. Friedhoff (Raven Press, New York) p. 149.

Young, A.B. and S.H. Snyder, 1973, Strychnine binding associated with glycine receptors of the central nervous system, Proc. Nat. Acad. Sci. U.S.A. 70, 2832.