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Effect of Kynurenine and Quinolinic Acid on the Action of Convulsants in Mice

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LAPIN, I. P. *Effect of kynurenine and quinolinic acid on the action of convulsants in mice.* PHARMAC. BIOCHEM. BEHAV. 13(1) 17–20, 1980.—Intraventricular injection of DL-kynurenine and L-kynurenine sulfate (40 μg) into conscious mice potentiated convulsions and lethality produced by strychnine (1 mg/kg) and not by thiosemicarbazide nor pentylenetetrazol. Another metabolite of tryptophan with convulsive effect, quinolinic acid, was ineffective. Intraperitoneal injection of DL-kynurenine sulfate and quinolinic acid (25–100 mg/kg) was associated with prolongation of the latency of strychnine and thiosemicarbazide (only the former drug) seizures. Nicotinic, picolinic, and anthranilic acids (100 and 250 mg/kg) did not modify the action of convulsants. Data and suggestions about probable involvement of brain glycine and gamma-aminobutyric acid receptors in the convulsive action of kynurenines is discussed.

Kynurenine Quinolinic acid Strychnine Thiosemicarbazide Pentylenetetrazol Seizures

RECENT findings have shown that metabolites of tryptophan on the kynurenine pathway (kynurenines) possess convulsive action in frogs [4], mice [9] and rats [10]. Kynurenine is synthethised in the human [5] and rat [2,5] brain. It penetrates into the mouse brain when injected intraperitoneally [3]. It has been suggested that increased concentrations of brain kynurenines might be involved in the genesis of seizures [9]. We, therefore, attempted to study whether kynurenines modify the action of convulsants and, if so, whether there is any convulsant, whose action is particularly affected by kynurenines. Expected similarity with a drug with a postulated mechanism of action seemed to help in understanding the mechanism of action of kynurenines. Among kynurenines we took two, kynurenine (K) and quinolinic acid (QA), which possess the strongest and most reliable convulsive effect [9] and are used for this reason in our studies as standard convulsive kynurenines. Other kynurenines in a series of experiments were used to compared them in general with K and QA.

METHOD

Animals

Albino SHR mice (bred from Swiss) of both sexes weighing 18–22 g from the Rappolovo farm were used. Before injections mice were kept in groups of 10–12 animals in metallic boxes (20×15×10 cm) and after administration of convulsants in groups of 4 animals in the same boxes.

Drugs

DL-K and L-K sulfates, QA and other kynureines (anthranilic, picolinic, nicotinic acids) in water solutions were injected intraperitoneally (IP) in a volume of 0.01 ml/g. Controls were treated with the same volume of distilled water. Solutions of kynurenines were injected into conscious mice by means of a semiautomatic apparatus [13] in a volume of 0.005 ml into the right cerebral ventricle. Controls were treated with the same volume of saline. Accuracy of injections was tested in a special group of controls in each experiment by means of a 0.25% solution of methylene blue. The stain was distributed homogeneously in the ventricular system in 80–95 percent of the mice. Kynurenines were administered IP 30 min prior to and intraventricular 5 min prior to these convulsants: strychnine sulfate, thiosemicarbazide, and pentylenetetrazol, which were injected subcutaneously (SC). Doses of all drugs used are given for salts.

Procedure

The following parameters of the convulsant action were registered: clonic convulsions (CC), their latency (in min), tonic extension (TE), its latency (in min), lethality (L), and survival time (in min). After injection of strychnine and pentylenetetrazol observation lasted 40 min, and after thiosemicarbazide 180 min. Room temperature was 19–21°C. Data on time were analyzed statistically by Student's t test, and on seizures and lethality by χ^2 test.

RESULTS

DL-K and, to an even greater degree, L-K potentiated seizures produced by strychnine while those produced by the two other convulsants, pentylenetetrazol and thiosemicarbazide remained unaffected (Table I). A potentiating dose of DL-K and L-K (40 μg) is high because it produced brief motor excitement in about half of the mice prior to injection of strychnine. CC were noted in some individuals. QA did not potentiate the action of any convulsant (Table 1) although its highest doses (1 and 2 μg) possess a much stronger stimulant action than the highest dose (40 μg) of DL-K and L-K. The same was true for the highest dose of nicotinic acid (40 μg), which produced motor excitement in half of the individuals but did not potentiate the action of strychnine.

TABLE 1

EFFECT OF DL- AND L- KYNURENINE SULFATE AND QUINOLINIC ACID INJECTED INTO BRAIN VENTRICLES ON THE ACTION OF CONVULSANTS

Drug intraventricular	μg	Effect of convulsants (SC) Number of mice Total	CC	TE	L
		Strychnine (1 mg/kg)			
Saline		12 M	1	1	1
DL-K	0.1	12 M	2	2	2
QA	0.1	12 M	1	1	1
Saline		6 M	1	1	1
DL-K	1.0	12 M	2	2	2
QA	1.0	12 M	5	5	5
Saline		12 F	0	0	0
DL-K	4.0	12 F	1	1	1
	40.0	12 F	10†	7†	7†
Saline		11 F	2	1	1
DL-K	40.0	11 F	9†	2	5
L-K	40.0	12 F	9†	7†	7†
QA	2.0	11 F	0	0	0
Saline		10 M	0	0	0
DL-K	40.0	10 M	4	1	2
L-K	40.0	10 M	8†	8†	8†
		Pentylenetetrazol (50 mg/kg)			
Saline		12 M	0	0	0
DL-K	1.0	12 M	0	0	0
QA	1.0	12 M	0	0	0
		Pentylenetetrazol (60 mg/kg)			
Saline		12 F	0	0	0
DK-L	40.0	12 F	3	1	1
L-K	40.0	12 F	4	1	1
Saline		12 M	10	10	10
DL-K	40.0	12 M	11	9	11
		Thiosemicarbazide (10 mg/kg)			
Saline		24 M	2	2	2
QA	1.0	12 M	1	0	0
	10.0	12 M	0	0	0
DL-K	2.0	12 M	4	2	2
	40.0	12 M	2	1	0
Saline		12 F	1	0	0
DL-K	40.0	12 F	3	0	2

Abbreviations: DL-K—DL-kynurenine sulfate, L-K—L-kynurenine sulfate, QA—quinolinic acid, M—male, F-female; CC—clonic convulsions, TE—tonic extension, L—lethality.

†$p<0.01$.

Picolinic (40 μg) and anthranilic (40 and 80 μg) acids did not potentiate the action of the threshold doses of strychnine.

When injected IP DL-K and QA in doses of 0.5–100 mg/kg did not modify the threshold dose of strychnine. DL-K and QA (50–100 mg/kg) prolonged the latency to strychnine seizures (Table 2). A similar effect was observed after NA (250 mg/kg). DL-K also diminished the rate of CC, TE and L (Table 2). L-K and PA in a dose of 100 mg/kg did not modify the action of strychnine.

Of all kynurenines tested IP, only DL-K and PA prolonged the latency to seizures produced by thiosemicarbazide (Table 2). The effect of DL-K was about the same at doses 0.5–100 mg/kg.

Neither kynurenine IP altered the action of pentylenetetrazol.

DISCUSSION

Enhancement by intraventricular DL-K and L-K of the convulsive action of strychnine and lack of increase of the action of the two other convulsants used speaks in favour of selectivity of interaction between DL-K and L-K and strychnine. Although the enhancement of strychnine seizures was observed after a high, exciting dose of DL-K and L-K, it is unlikely that this effect depends only on increased excitability because high doses of QA and NA stimulated animals even more markedly, but they did not enhance the action of strychnine. A suggestion of selectivity of interaction between DL-K and L-K and strychnine is in agreement with recent data on synergism of these drugs as well as reflections about similarity between them. L-K potentiates in vitro inhibition of uptake of serotonin in human blood platelets by convulsants, particularly by strychnine (in press). DL-K and L-K injected into brain ventricles lowered the threshold convulsive dose of strychnine in the frog (in press). L-glycine intraventricularly attenuated the convulsive action of L-K in mice much more than that of strychnine (in press). DL-K inhibits enzymes of metabolism of gamma-aminobutyric acid (GABA) of mouse brain (in press). The convulsive effect of L-K in mice is antagonized by GABA and its derivatives (in press). Data mentioned above have suggested (in press) that DL-K, L-K and some other kynurenines, according to the probable mechanism of their convulsive action, belong to the group of convulsants which inhibit inhibitory systems of the brain, e.g., strychnine, picrotoxin, penicillin, bicucullin, etc. Indeed, microinjection of DL-K into the frog hippocampus increased the epileptiform activity of the focus made in this structure by penicillin [4]. Because enhancement of the action of strychnine by DL-K and L-K was observed in the present study under intraventricular administration, one may suggest that synergism between these drugs occurs in brain structures adjacent to ventricles (e.g., hippocampus). There is no evidence so far to speculate on whether intraventricular kynurenines and strychnine are interacting at the level of the spinal cord. Inactivity of other kynurenines intraventricularly in enhancing the action of strychnine may be related to their poor penetration into these structures from ventricles and/or lack of their stimulant action in structures which are involved in the action of strychnine.

In the present study the convulsive action of DL-K and L-K (doses of 40 μg and higher, intraventricularly) as well as QA (2 μg and higher, intraventricularly) in mice was confirmed. This action of kynurenines may underly behavioral activation observed after tryptophan loading [11]. Which one of these two effects, behavioral depression or behavioral activation, will predominate after tryptophan loading may depend on the proportion of brain serotonin and brain kynurenines, respectively, formed from tryptophan. It has been reported [7,8] that these metabolites of tryptophan antagonize each other in a number of behavioral tests.

Taking into account the good penetration into the mouse brain of DL-K administered IP [3], even under doses of 0.5 and 5 mg/kg, one has trouble trying to understand why

TABLE 2

EFFECT OF DL- AND L-KYNURENINE SULFATE, QUINOLINIC ACID AND SOME OTHER KYNURENINES INJECTED INTRAPERITONEALLY ON THE ACTION OF CONVULSANTS

Drug IP	mg/kg	Effect of convulsants (SC) Number of mice Total	CC	TE	L	Latency to CC min mean ± SE
		Strychnine (1.5 mg/kg)				
D.W.	—	12 M	6	6	5	11.0 ± 0.6
DL-K	0.5	12 M	7	7	6	10.2 ± 0.9
QA	0.5	12 M	4	4	3	12.5 ± 0.3
D.W.	—	12 M	9	8	8	9.9 ± 0.4
DL-K	25.0	12 M	4	3*	2*	11.2 ± 1.2
	50.0	12 M	3*	3*	2*	12.6 ± 3.1
	100.0	12 M	7	7	5	14.4 ± 1.4†
QA	25.0	12 M	6	6	4	10.0 ± 1.6
	50.0	12 M	6	5	5	12.5 ± 1.0*
	100.0	12 M	7	7	5	17.3 ± 1.5‡
D.W.	—	11 M	11	11	11	11.1 ± 0.7
L-K	100.0	10 M	10	10	8	11.1 ± 0.9
D.W.	—	11 F	8	7	7	14.1 ± 1.2
NA	100.0	10 F	8	8	8	12.1 ± 1.1
PA	100.0	10 F	4	4	4	11.0 ± 1.0
D.W.	—	12 M	12	11	11	9.4 ± 0.4
NA	250.0	12 M	12	12	12	10.4 ± 0.6
D.W.	—	10 M	10	9	5	10.5 ± 0.8
AA	25.0	10 M	10	9	7	9.7 ± 0.4
	50.0	10 M	10	10	9	9.7 ± 0.6
	100.0	10 M	8	7	7	11.4 ± 0.6
D.W.	—	10 M	9	9	9	6.6 ± 0.5
DL-K	100.0	10 M	9	9	9	9.8 ± 1.3*
L-K	100.0	10 M	10	10	10	8.3 ± 0.7
QA	100.0	10 M	10	10	10	9.2 ± 0.7†
AA	250.0	10 M	10	10	10	9.2 ± 0.6†
		Thiosemicarbazide (10 mg/kg)				
D.W.	—	12 M	6	3	3	74.3 ± 2.9
DL-K	0.5	12 M	3	0	0	99.7 ± 2.7‡
	5.0	12 M	5	3	2	95.5 ± 22.9
		Thoisemicarbazide (20 mg/kg)				
D.W.	—	12 M	11	9	10	61.2 ± 2.5
DL-K	50.0	13 M	11	11	10	84.3 ± 3.4‡
QA	50.0	11 M	11	10	10	75.6 ± 6.8
D.W.	—	10 M	9	9	8	55.3 ± 5.9
DL-K	100.0	10 M	10	9	8	79.9 ± 3.9‡
QA	100.0	10 M	10	10	10	64.8 ± 3.4
D.W.	—	10 M	9	9	8	70.2 ± 4.1
L-K	100.0	10 M	10	10	9	70.9 ± 2.2
D.W.	—	10 F	10	10	9	74.0 ± 2.6
NA	100.0	10 F	4	4	4	70.5 ± 11.1
PA	100.0	10 F	7	7	6	93.4 ± 5.0†
		Pentylenetetrazol (60 mg/kg)				
D.W.	—	12 M	2	1	1	10.5 ± 3.5
DL-K	0.5	12 M	4	0	0	17.2 ± 4.3
	5.0	12 M	5	1	1	14.8 ± 3.7

TABLE 2
cont.

		Pentylenetetrazol (80 mg/kg)				
D.W.	—	10 M	5	0	0	12.7 ± 4.4
DL-K	50.0	10 M	6	0	0	9.1 ± 1.0
QA	50.0	10 M	6	1	1	14.0 ± 2.8
D.W.	—	10 M	7	1	1	15.4 ± 1.9
DL-K	100.0	10 M	6	1	1	18.0 ± 4.3
L-K	100.0	10 M	7	4	3	18.4 ± 2.1
QA	100.0	10 M	9	3	1	15.0 ± 1.7
D.W.	—	20 F	15	10	10	12.2 ± 2.6
DL-K	100.0	10 F	6	1	1	12.7 ± 3.1
L-K	100.0	10 F	7	5	5	16.3 ± 2.4
QA	100.0	10 F	9	5	5	18.0 ± 4.0
PA	100.0	20 F	19	14	14	14.1 ± 1.4
NA	100.0	20 F	10	4	3	17.0 ± 3.3
AA	100.0	10 F	9	2	2	9.1 ± 1.0

Abbreviations: see Table 1; NA—nicotinic acid, PA—picolinic acid, AA—anthranilic acid, D.W.—distilled water.
*$p<0.05$; †$p<0.01$, ‡$p<0.001$.

DL-K and L-K at doses 0.5–100 mg/kg, IP, used in the present study did not enhance the action of strychnine. It seems logical that doses of 25–100 mg/kg can increase brain concentration of DL-K and L-M much greater than doses of 0.5 and 5 mg/kg. Perhaps the increase does not occur in sufficient amounts in structures which are involved in the action of strychnine and which are reached easily by DL-K and L-K from brain ventricles.

Prolongation of the latency to strychnine seizures after IP injection of DL-K and QA and not by L-K, as shown here and in our experiments with intraventricular administration of a more active metabolite than DL-K, seems to be puzzling. This effect is reproducible, mild and dose-dependent. Of other kynurenines tested only AA in a dose of 250 mg/kg, i.e. 2.5–5 times higher than active doses of DL-K and QA, had a similar effect. Prolongation of the latency to strychnine seizures is related presumbly to peripheral actions of DL-K and QA.

No less puzzling appeared to be the prolongation of the latency to thiosemicarbazide seizures after IP injection of DL-K at doses of 0.5–100 mg/kg. This effect was not dose-dependent. Again, as in the case of the latency to strychnine seizures, L-K was inactive. The only point we know about the prolongation of the latencies to both strychnine and thiosemicarbazide seizures is that it is related to neither the general inhibitory action of DL-K (or QA and AA) nor hypothermia because DL-K in the doses used did not decrease locomotion and rearings and did not lower rectal temperature in mice.

The convulsive action of pentylenetetrazol was not affected by either kynurenine intraventricularly or IP. Whether this is related to the fact that brain serotonin does not play any significant role in the convulsive action of pentylenetetrazol in mice [6,12] remains unknown. In the rat, however, serotoninergic drugs modify seizures produced by pentylenetetrazol [1].

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