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GLYCINE POTENTIATES THE ACTION OF SOME ANTICONVULSANT DRUGS IN SOME SEIZURE MODELS

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The anticonvulsant effect of either phenobarbital or dilantin was potentiated by exogenous glycine in DBA/2 audiogenic seizure mice and in 3-mercaptopropionic acid-induced seizures. In seizures caused by pentylenetetrazol, glycine potentiated the anticonvulsant effect of phenobarbital only slightly; in combination with dilantin, which was ineffective by itself, it did not have an effect. Valproic acid, in large doses, prevented 3-mercaptopropionic acid-induced seizures; glycine did not potentiate its effect. Glycine thus potentiates anticonvulsant effects, but only of some drugs and only in some of the seizure models. This suggests that the mechanism of the anticonvulsant effect of glycine is similar to that of some of the anticonvulsant drugs such as dilantin and different from others, and that this mechanism is not effective in all seizure models.

INTRODUCTION

We have shown previously that systemic administration of large doses of glycine results in an elevation of its cerebral level and that this provides significant protection against some of the chemically- and sound-induced seizures in mice (1). Glycine administration to rats also provides protection against hyperbaric oxygen- and thiosemicarbazide-induced seizures (2). Since the cerebral glycine levels were not altered in the 3-mercaptopropionic acid (MPA) seizure models just before the convulsions started (3) and the glycine content of brain was slightly higher in the DBA/2 audiogenic seizure-susceptible than in the BALB/cBy audiogenic seizure resistant mice (4), it seems likely that the mechanism of anticonvulsant effect of glycine is not merely a replenishment of an induced or inherited

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general glycine deficiency in the brain. However, it can not be excluded that the glycine levels of some glycinergic neurons are significantly lower in these seizure model mice than in normal animals.

To investigate the mechanism of the anticonvulsant effect of glycine, we examined whether glycine can amplify the effect of anticonvulsant drugs such as phenobarbital, dilantin, and valproic acid in various animal seizure models. Phenobarbital was shown earlier to prevent xenogenic seizures in mongolian gerbil (5). In this report we demonstrate that phenobarbital and dilantin at higher dose prevent some chemically- and sound-induced convulsions and at lower dose levels their potencies are amplified in some seizure models by exogenous glycine administration.

EXPERIMENTAL PROCEDURE

Laboratory Animals. Adult BALB/cBy mice were bred in our animal colony. DBA/2 audiogenic seizure-susceptible mice (21–23 days old) were obtained from Jackson Laboratory, Bar Harbor, Maine. The animals were fed with standard diet and water ad libitum.

Chemicals. Dilantin (diphenylhydantoin) was from Parke-Davis, Detroit, Michigan. Phenobarbital (5-ethyl-5-phenyl-2,4,6-trioxohexahydropyrimidine), 3-mercaptopropionic acid, pentylenetetrazol (6,7,8,9-tetrahydro-5-H-tetrazolo-[1,5-a]azepine;α,β-cyclo-pentamethylenetetrazole), and valproic acid (2-propyl-pentanoic acid) were purchased from Sigma, St. Louis, Missouri. Glycine (ammonium-free) was obtained from Calbiochem, La Jolla, California.

Administration of Chemicals. Since glycine penetrates the brain slowly it was administered intragastrically (4) from a 2 M solution at 0 time. Dilantin, phenobarbital, or valproic acid was injected i.p. at 50 min and the convulsant was injected at 60 min, also i.p.

Seizure Models

Audiogenic Seizure-Susceptible DBA/2 mice. The seizure susceptibility was tested 10 min after the injection of dilantin or phenobarbital. The tests were performed by placing the animals in a 45 × 30 cm jar with a 5″ electric door-bell mounted on top (1).

3-Mercaptopropionic Acid-Induced Seizure. The MPA solution (1.22 g/ml) was diluted with 0.9% NaCl solution daily to 3 mg/ml and a 50 μg/g dose was injected per mouse, 16–20 weeks old.

Pentylenetetrazol (PTZ)-Induced Seizure. PTZ was injected in 0.9% NaCl solution. The dose was 90 μg/g of body weight. The animals were observed and the type and time of onset of seizures were noted.

RESULTS

Glycine, phenobarbital, and dilantin all provided significant protection for the DBA/2 audiogenic seizure mice in larger doses, 150 μmol/g, 50 μg/g, and 20 μg/g respectively (Table I). The combined administration of a smaller dose (2 μg/g) of phenobarbital, which alone had only slight

TABLE I

EFFECT OF GLYCINE ON THE ANTICONVULSANT ACTIVITY OF PHENOBARBITAL AND DILANTIN IN DBA/2 AUDIOGENIC SEIZURE MICE

Drug	Dose per g of body weight	N	Onset of seizures, min	N_1	N_2	N_3	N_4	N_1 percent	N_2 percent	N_3 percent	N_4 percent
—	—	16	2 ± 1	16	15	14	14	100	94	88	88
Glycine	150 μmol	9	6.8 ± 2	4	2	1	1	44	22	11	11
"	50 μmol	7	4 ± 3	7	7	7	6	100	100	100	86
Phenobarbital	50 μg	3		0	0	0	0	0	0	0	0
"	5 μg	3		3	1	0	0	100	33	0	0
"	2 μg	7	3 ± 1	7	7	6	5	100	100	86	71
Glycine + Phenobarbital	50 μmol 2 μg	13	6.0 ± 3	12	9	6	4	92	69	46	31
Dilantin	20 μg	3	7.0 ± 2	3	0	0	0	100	0	0	0
"	5 μg	7	3.0 ± 1	7	6	5	5	100	86	71	71
Glycine + Dilantin	50 μmol 5 μg	13	6 ± 3	12	7	3	2	92	54	23	15

Male DBA/2 mice, 22–26 days old, received glycine or 0.9% NaCl intragastrically at 0 time. Phenobarbital or dilantin was injected i.p. after 5 min, and the mice were tested for seizure susceptibility 10 min later. N = number of mice tested; N_1 = mice with wild running; N_2 = mice with clonic seizures; N_3 = mice with tonic seizures; N_4 = mice that died.

anticonvulsant effect, and a smaller dose (50 μmol/g) of glycine, also without anticonvulsant effect by itself, resulted in increased seizure protection in the audiogenic seizure mice. The number of mice with clonic and tonic seizures and the number of deaths were reduced by 31–40%. The anticonvulsant effect of a small dose of dilantin was also increased by glycine. The incidence of clonic seizures was reduced by 32%, of tonic seizures by 48%, and of deaths by 56% (Table I).

Glycine, phenobarbital, and dilantin had anticonvulsant effect also in the 3-mercaptopropionic acid-caused seizures at larger doses, and the anticonvulsant effect of each drug when given at a lower dose was amplified by glycine (Table II). The number of mice having clonic or tonic seizures after receiving 10 μg/g of phenobarbital was reduced by 29% and 86% respectively when glycine (25 μmol/g) was also given. Dilantin in 10 μg/g dose had no anticonvulsant effect, but when administered together with 25 μmol/g of glycine the incidence of clonic and tonic seizures was reduced by 50–100%. Glycine alone in 25 μmol/g dose reduced the number of tonic seizures by 15%, but it had no effect on the clonic seizures.

Valproic acid in 1 mg/g dose blocked both the clonic and tonic seizures induced by 3-mercaptopropionic acid, but the anticonvulsant effect of a

TABLE II

EFFECT OF GLYCINE ON THE ANTICONVULSANT ACTIVITY OF PHENOBARBITAL AND DILANTIN IN 3-MERCAPTOPROPIONIC ACID (MPA)-INDUCED CONVULSIONS

Drug	Dose per g of body weight	N	Onset of seizures, min	N_1	N_2	N_1 percent	N_2 percent
	—	8	4.5 ± 0.5	8	8	100	100
Glycine	50 μmol	4	7.0 ± 1.0	2	2	50	50
"	25 μmol	7	4.5 ± 1.3	7	1	100	15
Phenobarbital	50 μg	4	—	0	0	0	0
"	20 μg	4	12 ± 2.8	1	1	25	25
"	10 μg	7	7.5 ± 2.0	6	6	86	86
Glycine + Phenobarbital	25 μmol 10 μg	7	8.5 ± 2	4	0	57	0
Dilantin	20 μg	5	19	1	0	20	100
"	10 μg	8	4.5 ± 0.5	8	8	100	
Glycine + Dilantin	25 μmol 10 μg	8	5.3 ± 1.5	4	0	50	0

BALB/cBy mice, 4–5 months old, received glycine intragastrically and after 50 min phenobarbital or dilantin by i.p. injection. MPA 50 μg/g was injected i.p. 10 min after phenobarbital or dilantin. N = number of mice tested; N_1 = mice with clonic seizures; N_2 = mice with tonic seizures.

smaller dose (200 μg/g) of valproic acid, which provided little protection alone, was not amplified by glycine (data not shown).

In the pentylenetetrazol treated animals only phenobarbital had a significant anticonvulsant effect. Glycine had a weak effect, and dilantin had no anticonvulsant effect (Table III). A dose of dilantin higher than 150 μg/g was lethal to the animals. The combined treatment of mice with 5 μg/g of phenobarbital and 25 μmol/g of glycine resulted in increased protection against tonic seizures and death, but it had no effect on clonic seizures. The combined administration of dilantin and glycine did not result in any anticonvulsant protection in these animal (Table III).

DISCUSSION

The mechanism of anticonvulsant drugs in preventing or diminishing seizures is not clearly established. Among the many anticonvulsant drugs, there is none which is effective against all types of seizures. This indicates that there are most likely several mechanisms of action for these drugs. Neuronal excitability can be diminished by increasing inhibitory or decreasing excitatory transmission. The former can take place if the levels

TABLE III

EFFECT OF GLYCINE ON THE ANTICONVULSANT ACTIVITY OF PHENOBARBITAL AND DILANTIN IN PENTYLENETETRAZOL-INDUCED CONVULSIONS

Drug	Dose per g of body weight	N	Onset of seizures, min	N_1	N_2	N_3	N_1 percent	N_2 percent	N_3 percent
—	—	7	2.1 ± 1.2	7	7	6	100	100	86
Glycine	50 μmol	3	1.6 ± 0.1	3	1	1	100	33	33
"	25 μmol	4	1.0 ± 0.4	4	4	3	100	100	75
Phenobarbital	50 μg	4	3.4	1	0	0	25	0	0
"	10 μg	4	1.9 ± 0.9	4	0	0	100	0	0
"	5 μg	4	1.9 ± 1	4	4	4	100	100	100
Glycine + Phenobarbital	25 μmol 5 μg	8	2.0 ± 1.3	8	6	2	100	75	25
Dilantin	150 μg	4	2.6 ± 0.3	4	4	1	100	100	25
"	20 μg	4	2.7 ± 0.3	4	4	4	100	100	100
Glycine + Dilantin	25 μmol 20 μg	4	3.0 ± 0.8	4	4	4	100	100	100

BALB/cBy mice, 4–5 months old, received glycine intragastrically and after 50 min phenobarbital or dilantin intraperitoneally. Pentylenetetrazol 90 μg/g was injected i.p. 10 min after phenobarbital or dilantin. N = number of mice tested; N_1 = mice with clonic seizures; N_2 = mice with tonic seizures; N_3 = mice that died.

of inhibitory neurotransmitters increase and the latter occurs if the levels of excitatory neurotransmitters are depleted or their site of action is blocked. A number of studies raised the possibility that the mode of action of the anticonvulsant drugs is through their effect on the metabolism of amino acid neurotransmitters, especially GABA (6–8). Prolonged treatment with phenobarbital or valproic acid results in the elevation of GABA levels in several brain regions of the rat (7, 8). Acute valproic acid treatment also increases GABA levels and reduces the level of aspartic acid in rat brain (9). Seizures produced by GAD inhibitors are blocked by phenobarbital (10, 11). It has been postulated that phenobarbital acts primarily at the bicuculline-sensitive GABA receptor site (12, 13). Dilantin increases GABA levels (8, 14) and provides protection against seizures induced by various GAD inhibitors (10, 15). The uptake of GABA into synaptosomes is increased by dilantin (16). Anticonvulsants have other effects not related to GABA. Spontaneous or potassium-stimulated efflux of D-aspartate from rat brain minislices is depressed by phenobarbital (17). At high concentration phenobarbital inhibits voltage-dependent calcium entry into synaptosomes (18, 19). Dilantin affects neurotransmitter systems, such as norepinephrine (16, 20), dopamine (21), and serotonin (22), and the active transport of sodium (23) and calcium (18) ions and

the activity of Na^+-K^+-ATPase (24). It has been shown more recently that dilantin inhibits calcium, calmodulin-regulated protein phosphorylation in neuronal preparations (25) and displaces diazepam from benzodiazepine receptors (26).

It was found that valproate medication in children increased the level of glycine in the serum and in the urine, but the CSF levels of those examined were normal (27). In a study of the effects of three anticonvulsants in children, only valproic acid increased plasma glycine levels; phenobarbital and carbamazepine had no effects (28). The mechanism of action of valproate may occur through inhibition of glycine catabolism. The activity of the glycine cleavage system, the major pathway for glycine catabolism, was reduced in liver homogenates of valproic acid-treated rats; glycine cleavage was also inhibited by valproate added in vitro (29). These findings do not necessarily indicate an increase of glycine in the brain since glycine penetration through the blood-brain barrier is slight. There were only a few measurements of brain glycine following anticonvulsant administration. Di-*n*-propylacetate given intraperitoneally to rats increased brain GABA content but had no effect on brain glycine levels (30).

The findings that glycine has an anticonvulsant effect in 3-mercaptopropionic acid (MPA)-induced seizures, caused by a cerebral GABA deficit (10, 11), and also in DBA/2 audiogenic seizure mice, which show no GABA deficit, and that glycine reduces the level of glutamic acid (1) and slightly increases GABA in the seizure mice (unpublished observation), may be interpreted as showing that the mechanism of anticonvulsant effect of glycine is similar to that of some anticonvulsant drugs, by influencing GABA or glutamate levels.

The combined administration of glycine and phenobarbital or dilantin to DBA/2 audiogenic seizure mice and to those with MPA-induced seizures results in increased seizure protection (Table I, II). Glycine is not effective in all cases. In pentylenetetrazol-induced seizures, where phenobarbital has an anticonvulsant effect and dilantin does not, glycine slightly potentiated the effect of phenobarbital but not that of dilantin. The anticonvulsant effect of valproic acid also is not potentiated by glycine in the MPA-induced seizures, although valproic acid itself in large doses did prevent these seizures. Since valproic acid increased brain GABA levels, as did phenobarbital (7, 17), and glycine did not potentiate the effect of valproic acid but increased that of phenobarbital in the MPA models, and glycine itself provided significant seizure protection in this model, the mechanism of the anticonvulsant effect of glycine and of the tested anticonvulsant drugs probably involves another neurotransmitter

system(s), not only the GABAergic system. It is possible that some of the anticonvulsant drugs and glycine act through the glycinergic neurons.

We did not measure drug levels in the plasma and brain, therefore it can not be excluded that glycine affects their metabolism. Since some seizure disorders in humans are detectable only with electroencephalography and do not show physical seizure activity (31), it is possible that although the physical manifestation of seizures was suppressed in the mice by glycine some abnormal electrical activity was still present in the brain.

The observed potentiating effect of glycine on some anticonvulsant drugs indicates the need for further studies, including one on the applicability of results with rodents to human pathology, concerning the therapeutic use of glycine in some seizure disorders.

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