12. T. W. Nilsen and C. Baglioni, *Proc. Nat. Acad. Sci. USA*, **76**, № 6, 2600-2604 (1979).
13. T. W. Nilsen, P. A. Maroney, and C. Baglioni, *J. Virol.*, **42**, 1039 (1982).
14. S. Pestka, J. A. Langer, K. Zoon, and C. E. Samuel, *Ann. Rev. Biochem.*, **56**, 727-777 (1987).
15. N. B. K. Ray and P. M. Pitha, *Proc. Nat. Acad. Sci. USA*, **80**, 3923-3927 (1983).
16. B. Y. Rubin and S. L. Gurta, *J. Virol.*, **34**, № 2, 446-454 (1980).
17. R. M. Silverman, P. J. Cayley, M. Knight, *et al.*, *J. Biochem.*, **124**, 131 (1982).
18. W. E. Stewart II, *The Interferon System*, Springer-Verlag, Vienna-New York (1979).
19. C. Weil, C. Y. Epstein, and L. B. Epstein, *Nature*, **301**, № 5899, 437-439 (1983).
20. B. R. G. Williams, C. S. Gilbert, and I. M. Kerr, *Nucl. Acid Res.*, **6**, 1335-1350 (1979).
21. Y. K. Yamada, A. Meager, A. Yamada, and F. A. Ennis, *J. Gen. Virol.*, **67**, № 11, 2325-2334 (1986).

Anticonvulsive Activity of Glutapyrone, a New Type of Derivative of Amino Acid-Containing 1,4-Dihydropyridines

M. N. Karpova, S. K. Germane, G. Tsebers, O. Yu. Pankov, V. E. Klusha, G. Ya. Duburs, and E. A. Bisenieks

UDC 615.213.038].07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 9, pp. 283-286, September, 1993
Original article submitted May 26, 1993

Key Words: *1,4-dihydropyridine; glutapyrone; epileptic activity; corasole; $^{45}Ca^{2+}$ uptake*

Screening new drugs with anticonvulsive and antiepileptic effects is of great importance, since the diversity of clinical manifestations of epilepsy calls for equally diverse actions of drug preparations.

A new compound, synthesized at the Latvian Institute of Organic Synthesis, disodium salt (2-2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridyl-4-carboxamide) of glutaric acid, comprises structures of both 1,4-dihydropyridine (DHP) and glutamic acid bound as sodium salt to the 4th position of the DHP ring. This compound differs greatly from classical DHP in its being highly soluble in water, in its low toxicity (LD_{50}>8000 mg/kg, i.p.), and in its exhibiting a pronounced and prolonged antiarrhythmic [9] and stress-protective effect [7].

Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow; Latvian Institute of Organic Synthesis, Riga. Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

The present study is devoted to an investigation of the effect of glutapyrone on $^{45}Ca^{2+}$ uptake by synaptosomes of the cerebral cortex of rats, on acute corasole-generalized seizures, and on arecoline and nicotine tremor.

MATERIALS AND METHODS

The experiments were carried out on 211 male Wistar rats weighing 210-260 g and on 300 male Icr:Icl mice weighing 18-25 g. The animals were kept in the vivarium under the usual conditions and on a standard diet. $^{45}Ca^{2+}$ uptake by the synaptosomes of the cerebral cortex was studied according to Pellmar and Wilson's method [8] slightly modified by us [3]. Acute generalized epileptic activity was provoked in the rats by intraperitoneal injections of corasole in a dose of 80 mg/kg. The effects observed were visually recorded over 60 min. The latent time of the first seizure manifestations and the latent time and duration of the clonic and tonic phases (falling of the animal

0007-4888/93/0009-1119$12.50

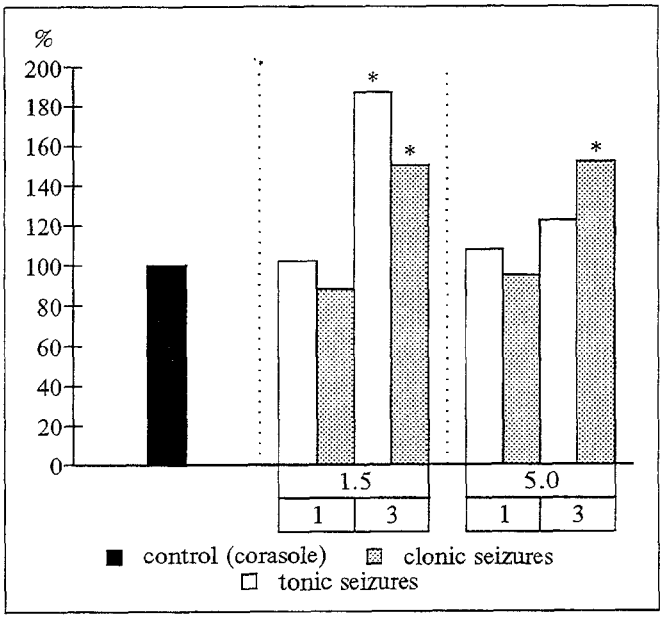

Fig. 1. Effect of glutapyrone on seizures induced in mice for intravenous titration. Ordinate: intensity of antiepileptic effect, %. The values of the same parameters in the control group are taken as 100%. Asterisk indicates $p<0.05$.

on its side) of the generalized seizure response, the severity of the seizures, the longevity, and the mortality were determined. The severity of seizures was assessed in points: clonic seizures as 1, repeated clonic seizures as 2, tonic-clonic seizures with falling on the side as 3, and repeated tonic-clonic seizures and/or death of the animal as 4 points. Six experimental series were performed. Glutapyrone was dissolved in physiological saline and administered in doses of 5, 10, and 20 mg/kg 1 and 3 h before the injection of corasole. In mice the seizures were induced by intravenous injection of a 1% solution of corasole at a rate of 0.01 ml/sec. The thresholds of clonic seizures and of tonic convulsive fits with a lethal outcome were recorded. The threshold dose of corasole required for provoking such seizures was calculated in mg/kg for each animal. Glutapyrone was administered in doses of 1.5 and 5 mg/kg i.p. 1 and 3 h before examination. Sodium valproate in doses of 0.5, 5, and 50 mg/kg was used as a reference anticonvulsant preparation. Control animals received physiological saline. The antitremorogenic activity was determined in the arecoline and nicotine tremor test. Arecoline was subcutaneously injected in a dose of 25 mg/kg, and the duration of tremor (min) and the presence of salivation and diarrhea were recorded. Nicotine (0.01% solution) was intravenously injected at a rate of 0.01 ml/sec. The dose of nicotine was calculated in mg/kg for each animal. The results were statistically processed using the parametric and nonparametric Student and Fisher tests.

RESULTS

Acute generalized seizures. Administration of 5 mg/kg glutapyrone 1 and 3 h before injection of corasole exerted no effect on the generalized seizure response (Table 1). When administered 1 h prior to corasole, glutapyrone in a dose of 10 mg/kg delayed the development of clonic seizures and reduced the number of animals dying. Administration of the preparation in the same dose 3 h before corasole injection increased the latent period of the first manifestations of seizures, delayed the development of clonic and tonic seizures, extended

TABLE 1. Effect of Glutapyrone on Generalized Epileptic Activity in Rats ($M\pm m$)

Series	Group and number (n) of animals	Dose and time before injection of corasole	LP 1, sec	Clonic phase		Tonic phase		Time of death, sec	Number of dead animals	Severity of seisures, points
				LP 2, sec	duration, sec	LP 3, sec	duration, sec			
1	Control ($n=12$)		43.3±3.9	54.5±5.5	20.1±1.8	192.0±28.9	16.4±1.7	577.1±195.1	7	2.92±0.26
	Exp. ($n=12$)	5 mg/kg, 1 h	37.2±2.6	41.9±3.1	20.3±2.9	316.2±93.4	16.8±2.3	491.4±127.9	6	2.83±0.26
2	Control ($n=13$)		33.3±2.5	39.8±3.3	21.5±1.2	112.5±11.8	15.9±2.0	276.0±18.3	9	3.23±0.25
	Exp. ($n=12$)	5 mg/kg, 3 h	34.2±3.7	40.3±5.4	20.3±2.2	108.8±13.6	12.3±1.1	254.16.5	10	3.67±0.26
3	Control ($n=13$)		28.3±3.1	37.7±4.9	22.7±1.9	93.8±11.9	16.8±1.4	729.1±122.8	8	2.92±0.25
	Exp. ($n=13$)	10 mg/kg, 1 h	33.2±4.4	39.2±5.6	22.6±2.0	154.6±10.9 $p<0.01$	11.4±3.3	1122.5±412.8	2 $p<0.025$	2.00±0.25 $p<0.02$
4	Control ($n=13$)		27.5±2.2	33.2±2.7	22.1±1.8	95.0±6.5	16.9±0.9	220.4±18.2	10	3.69±0.17
	Exp. ($n=13$)	10 mg/kg, 3 h	36.6±2.8 $p<0.02$	42.0±2.3 $p<0.05$	22.7±1.3	139.2±12.2 $p<0.01$	16.4±1.0	550.9±109.6 $p<0.02$	8	3.05±0.24 $p<0.05$
5	Control ($n=12$)		40.6±3.8	53.5±6.5	30.6±5.6	99.7±18.9	18.8±2.0	403.7±77.1	10	3.67±0.18
	Exp. ($n=12$)	20 mg/kg, 1 h	61.6±9.0 $p<0.05$	35.7±4.2 $p<0.05$	25.0±3.5	132.9±15.9	18.6±2.6	810.7±84.9 $p<0.01$	10	2.97±0.25 $p<0.05$
6	Control ($n=14$)		26.1±1.4	28.9±1.7	24.1±2.6	102.3±11.2	19.9±0.9	368.5±48.8	14	4.00±0.00
	Exp. ($n=13$)	20 mg/kg, 1 h	30.2±2.0	36.9±2.5 $p<0.02$	24.1±1.8	96.0±6.6	18.0±0.8	489.7±140.0	9 $p<0.025$	3.23±0.25 $p<0.01$

Note. LP 1, LP 2, and LP 3: latent period of first manifestations of seizures, and of clonic and tonic phases of generalized seizure response.

the life of the animals, and reduced the severity of the seizure response. Administration of the preparation in a dose of 20 mg/kg 1 h before injection of corasole increased the latent period of the first manifestations of seizures, delayed the development of the clonic phase of the generalized seizure response, increased the life span of the animals, and reduced the severity of the seizure response.

Thus, glutapyrone in a dose of 10 mg/kg exhibited an antiepileptic activity with respect to acute generalized seizures in rats. Raising the dose to 20 mg/kg did not enhance the antiepileptic effect. The antiepileptic effect of the preparation was virtually the same whether it was given 1 or 3 h prior to corasole. In mice with corasole-induced generalized seizures, glutapyrone in doses of 1.5 and 5 mg/kg, injected 3 h before corasole, raised the threshold of clonic seizures 85.4 and 86.7%, respectively. When administered either 1 or 3 h ahead of corasole, glutapyrone had a protective effect against the clonic phase of seizures with a lethal outcome (Fig. 1). Sodium valproate in doses of 0.5-50 mg/kg did not produce any protective effect with respect to clonic seizures. However, against the background of sodium valproate, a considerably higher dose of corasole was required to provoke tonic seizures with a lethal outcome. Thus, the doses of glutapyrone which produced an anticonvulsive effect (0.5-5 mg/kg) were markedly lower than those of sodium valproate (5-50 mg/kg). In addition, sodium valproate failed to exhibit any protective effect with respect to clonic seizures. The study of the duration of anticonvulsive action of the drugs showed that the maximum effect of sodium valproate is attained after 1 h; it then drops gradually and completely disappears after 6 h. Under the same experimental conditions, the anticonvulsive effect of glutapyrone is preserved during 6 h.

Antitremorogenic activity. Glutapyrone virtually did not affect arecoline and nicotine tremor, that is, the M- and N-cholinergic processes, respectively.

Effect of glutapyrone upon $^{45}Ca^{2+}$ uptake in vitro. As is seen from Table 2, glutapyrone (1-100 μM) did not affect $^{45}Ca^{2+}$ uptake by rat cortex synaptosomes. Hence, glutapyrone, in contrast to other DHP, is not a Ca^{2+} antagonist.

Our findings provide evidence that the new compound glutapyrone, an amino acid-containing derivative of 1,4-DHP, exhibits a pronounced effect in the model of generalized seizures in rats and mice. The intensity of the antiepileptic effect depends on the dose of preparation and on the method of simulation of corasole-provoked seizures. The effect of corasole is attributed to depolarization of the neuron membrane [10], to disrupted Cl^--conductivity [8], and to disturbances of the inhibitory GABA-ergic effects [2,4,6]. Taking into account these and other data on the ability of glutapyrone to normalize the stress-induced decrease of the GABA content in the brain [7], we may assume that the GABA-ergic system participates in the realization of the anticonvulsive effects of glutapyrone. The antioxidant component of the glutapyrone effect may also play a role [1]. At the same time, in contrast to classical 1,4-DHP (nifedipine, nimodipine, etc.), glutapyrone does not act upon $^{45}Ca^{2+}$ uptake by synaptosomes. In addition, it exerts no effect on the N- and M-cholinergic processes.

TABLE 2. Effect of Glutapyrone and Nifedipine on $^{45}Ca^{2+}$ Uptake by Synaptosomes Isolated from the Rat Cerebral Cortex ($M \pm m$)

Concentration of preparation	$^{45}Ca^{2+}$ uptake, nmol/mg protein		Total uptake
	K^+ 5 mM	K^+ 55 mM	
Control	0.67±0.07	1.47±0.10	0.82±0.05
Glutapyrone			
10 nM	0.68±0.13	1.35±0.32	0.66±0.19
1 μM	0.70±0.23	1.48±0.32	0.78±0.09
100 μM	0.68±0.17	1.45±0.38	0.77±0.18
Nifedipine			
1 μM	1.08±0.08	2.16±0.07	1.08±0.01
10 μM	1.08±0.02	1.96±0.03	0.88±0.10
100 μM	1.09±0.05	1.55±0.06*	0.46±0.01*

Note. Asterisk: $p<0.05$ vs. control.

REFERENCES

1. L. Ya. Utno, Z. E. Lipsberga, A. A. Silova, *et al., Byull. Eksp. Biol. Med.*, **106**, № 11, 558-561 (1988).
2. A. G. Chwey, E. A. Swinyard, and H. H. Wolf, *J. Neurochem.*, **41**, 830-833 (1983).
3. V. Klusa, G. Duburs, S. Germane, *et al., Proc. Latv. Acad. Sci.*, № 9, 51-55 (1991).
4. R. L. Macdonald and J. L. Barker, *Neurology (Minneap.)*, **27**, 337 (1977).
5. W. B. Mandelson, P. Skolnic, J. V. Martin, *et al., Eur. J. Pharmacol.*, **104**, 181-183 (1984).
6. P. J. Marangos *et al., Life Sci.*, **24**, 851-857 (1979).
7. R. Muceniece, I. Liepa, M. Dambrova, et al., in: *Pharmacology of Novel Centrally and Peripherally Acting Drugs*, Riga (1991), pp. 51-52.
8. T. S. Pellmar and W. A. Wilson, *Science*, **197**, 912-914 (1977).
9. M. Veveris, H. Cirule, in: *Pharmacology of Novel Centrally and Peripherally Acting Drugs*, Riga (1991), p. 76.
10. T. L. Williamsen and W. E. Crill, *Brain Res.*, **116**, 217-229 (1976).