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Effects of Glutapyrone, a New Amino Acid-Containing 1,4-Dihydropyridine, on Focal Penicillin-Induced Epileptic Activity and on Bicuculline- or Thiosemicarbazide-Induced Convulsions

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Antiepileptic effects of 1,4-dihydropyridines (DHP) have been demonstrated on various animal models of epileptic activity (EpA) and in humans [1] but, being calcium antagonists, these compounds also affect the hemodynamics, which limits their usefulness. Although glutapyrone - a compound synthesized at the Latvian Institute of Organic Synthesis - contains a DHP ring, the sodium salt of glutamic acid attached at position 4 to its molecule makes it a new type of compound (both quantitatively and qualitatively), referred to as an amino acid-containing DHP. Compounds of this type are readily soluble in water, have a low toxicity (LD_{50}>8000 mg/kg intraperitoneally and orally), do not lower arterial pressure [7], and have been shown to exhibit peptide-like regulatory mechanisms of action [8,11]. In addition, glutapyrone has been found to have antiarrhythmic and antiischemic [5,7] as well as antioxidant properties [3].

In this work, we studied glutapyrone for its effects on focal penicillin-induced EpA in the cerebral cortex of rats and on the convulsions induced in mice by bicuculline or thiosemicarbazide.

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MATERIALS AND METHODS

For the study, 211 male Wistar rats weighing 210-260 g and 190 male Icr:Icl mice weighing 19-23 g were used. All animals were maintained in the

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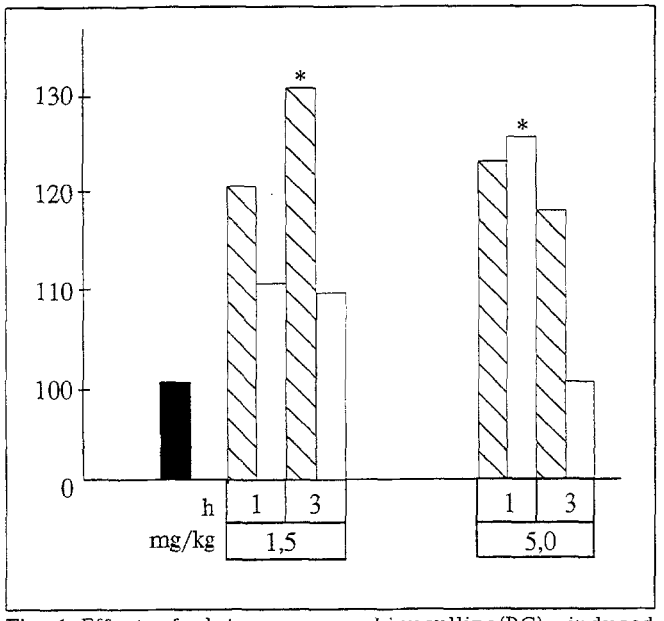

Fig. 1. Effect of glutapyrone on bicuculline(BC)-induced convulsions in mice. Ordinate: BC dose that led to clonic convulsions and mortality, expressed as % of its values in the control group taken as 100%. Dark bar: control (BC alone); hatched bars: clonic convulsions; white bars: mortality. Asterisks denote a statistically significant difference from the control ($p<0.05$).

vivarium under routine conditions and fed a standard diet. Focal EpA was produced in rats as described previously [2], leaving the dura mater intact. A focus of EpA was set up by applying to the sensorimotor cortex a piece of filter paper soaked in a solution of benzyl penicillin sodium (concentration 32,000 IU/ml). The electrocorticogram was recorded on an EEG 8S electroencephalograph (Hungary) in nonanesthetized (freely moving) animals. Glutapyrone was dissolved in physiological saline and injected intraperitoneally in a dose of 10, 30, or 60 mg/kg in the presence of stable EpA in the focus. The results were processed in an M-44 computer system (Olivetti, Italy). The amplitude-frequency characteristics of the foci and the duration of their existence were determined.

Convulsions were induced in mice by injecting them with a 0.01% bicuculline solution intravenously at a rate of 0.01 ml/sec until the occurrence of clonic and tonic convulsions leading to death. The bicuculline dose at which such convulsions appeared was expressed in mg/kg. Thiosemicarbazide was injected subcutaneously at 20 mg/kg either simultaneously with the test or reference compound or 2 h after their injection since the latent period before the emergence of thiosemicarbazide-induced convulsions is 1 h on average. As the reference anticonvulsant, sodium valproate was used in doses of 0.5, 5, and 50 mg/kg. The results were treated by Student's t test.

RESULTS

The application of penicillin to the sensorimotor area of the rat cortex resulted in EpA: 3-5 min postapplication solitary spike (interictal) discharges of progressively increasing amplitude appeared; 6-15 min later, convulsive, ictal discharges began to be recorded, followed, after annother 25-35 min, by the establishment of a pronounced and stable convulsive activity characterized by regular ictal discharges over a period of 30-40 min. Thereafter, these discharges were generated less and less

TABLE 1. Effect of Glutapyrone on Focal Penicillin-Induced Epileptic Activity (EpA) in Rats. The Values are Means±SEM

Group	Dose, mg/kg	Before glutapyrone injection			After glutapyrone injection			Duration of focus, min
		№ of IID per minute	IID amplitude, mV	№ of ID, per minute	№ of IID per minute	IID amplitude, mV	№ of ID, per minute	
Control ($n=8$)		18.77±2.13	1619±186	0.19±0.04	17.98±3.16	1597±0.06	0.18±0.06	145.13±14.25
Test ($n=8$)	10	19.54±1.87	1835±193	0.22±0.05	18.33±2.76	1713±194	0.20±0.08	142.67±18.46
Control ($n=8$)		19.73±1.76	1416±115	0.21±0.05	17.73±1.78	1251±104	0.17±0.05	139.50±11.17
Test ($N=8$)	30	17.36±1.44	1581±150	0.33±0.04	16.87±158	1595±158	0.10±0.04 $p<0.01$	126.75±9.43
Control ($n=8$)		19.20±4.61	1881±192	0.22±0.03	19.20±3.57	1614±175	0.20±0.07	140.80±16.51
Test ($n=10$)	60	18.16±1.76	2057±326	0.58±0.05	24.20±8.06	2069±307	0.22±0.09 $p<0.01$	71.75±9.60 $p^*<0.01$

Note. ID = ictal discharges; IID = interictal discharges; p: relative to the same group before glutapyrone injection; $^*p$: relative to the control group.

frequently, as were interictal discharges whose amplitude also steadily decreased. The mean period elapsing between penicillin application and the complete disappearance of EpA was 140±10 min. In rats of the control groups (Table 1), administration of physiological saline 25-30 min after penicillin (i.e., when ictal discharges were being generated in a stable manner) did not affect EpA in the focus.

Glutapyrone injected at 10 mg/kg in the presence of high ictal activity did not influence EpA. A dose of 30 mg/kg led to less frequent ictal discharges (Tables 1 and 2). Glutapyrone, however, did not affect the frequency or amplitude of interictal discharges, nor did it shorten the time during which the foci existed. The antiepileptic effect in animals of this group was most strongly marked at 30 min postinjection (Table 1) and lasted for 30 min. In two rats, EpA was completely suppressed 40 and 70 min, respectively, after glutapyrone administration. At the 60 mg/kg dose level, glutapyrone inhibited EpA activity in all rats, which was manifested in less frequent generation of ictal discharges and in reduced duration of the foci (Table 1).

In the tests with mice, glutapyrone was effective in much lower doses. At 1.5 and 5 mg/kg dose levels, it exhibited antiepileptic activity with respect to bicuculline-induced convulsions, particularly when injected 3 h before the convulsant (Fig. 1); in such rats, the threshold for tonic convulsions increased by 48.1 to 89.6%. Some antiepileptic activity was still evident after 24 h. Sodium valproate failed to exhibit a protective effect under such experimental conditions.

Thiosemicarbazide triggered the first convulsive attack 62.5±1.9 min postinjection, the second attack 20.3±3.8 min later, and death at 70.7±13.3 min postinjection (Fig. 2). Glutapyrone at 1.5 and 5 mg/kg prolonged by 22.7 and 30.7%, respectively, the latent period before the onset of the first convulsive attack and increased the survival of mice 1.7 times and 2.6 times, respectively; the period elapsing between the first and second attacks did not change significantly. Sodium valproate again did not exhibit antiepileptic activity under such experimental conditions.

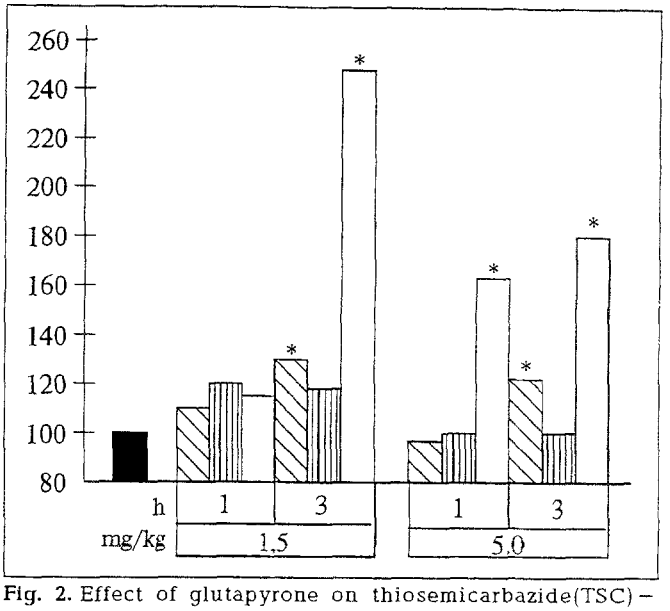

Fig. 2. Effect of glutapyrone on thiosemicarbazide(TSC)-induced convulsions in mice. Ordinate: TSC dose that led to convulsions, expressed as % of its values in the control group, taken as 100%. Dark bar: control (TSC alone); obliquely hatched bars: latent period before onset of first attack; vertically striped bars: latent period before onset of second attack; light bars: survival time. Asterisks denote a significant difference from the control ($p<0.05$).

The results of this study indicate that glutapyrone exerts a pronounced antiepileptic effect both in rats with focal penicillin-induced EpA and in mice with bicuculline- or thiosemicarbazide-induced convulsions. In our earlier study (in press), glutapyrone was also found to be effective in corazole-induced convulsions. The convulsants used in these two studies each impair a distinct mechanism of GABA-ergic inhibitory control. Thus, penicillin selectively antagonizes GABA-mediated inhibition [6] and decreases the number of GABA receptors [4,6]; the convulsive effects of thiosemicarbazide are due to the inhibition of glutamate decarboxylase activity followed by a reduction of GABA synthesis in the brain [10,13]; bicuculline competitively blocks GABA binding to its receptors [14], while corazole blocks GABA's action on the passage of Cl^- ions in membrane channels [9]

TABLE 2. Effect of Glutapyrone at 30 mg/kg on Ictal Discharge Generation in the Focus in Rats. The Values are Means±SEM

Group	№ of ID per minute before glutapyrone	№ of ID per minute after glutapyrone					
		1-10 min	10-20 min	20-30 min	30-40 min	40-50 min	50-60 min
Control ($n=8$)	0.21±0.05	0.13±0.05	0.18±0.05	0.17±0.05	0.20±0.05	0.13±0.05	0.23±0.08
Test ($n=8$)	0.33±0.04	0.15±0.04	0.11±0.04	0.10±0.04	0.15±0.04	0.17±0.05	0.08±0.05
		$p<0.02$	$p<0.01$	$p<0.01$	$p<0.02$	$p<0.05$	$p<0.01$

Note. ID = ictal discharges; p is given relative to the same group before glutapyrone injection.

and has a direct excitatory influence on neuron membranes [12]. Our studies (in press) have shown that glutapyrone, although a derivative of 1,4-DHP, does not block voltage-dependent Ca channels. It seems likely that this compound realizes its anticonvulsive activity predominantly via the GABA-ergic mechanisms.

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Elimination of Stress-Induced Changes in the Thymus within Laser Irradiation of the Endocrine Glands

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Adaptation medicine, a new scientific discipline, studying the mechanisms of adaptation of the organism to the environment or to the behavior of the organism itself and elaborating adaptive methods for the prevention and treatment of diseases has been formed in recent years [8]. One of the main aspects of this trend is the use of the adaptation for the prevention and treatment of stress-induced disorders.

The possibility of using low-energy laser radiation (LLR) in the infrared (IR) range as a stress-limiting factor needs to be explored. IR LLR (0.89 m) is a physical agent widely used at present and, unlike laser radiation of the visible part of the spectrum, it offers greater depth of penetration in tissues (of the order of 5-6 cm) and thereby the possibility of direct contact not only with the skin but with the deeper-lying tissues as well [5,6]. The feasibility of using of IR laser radiation to correct cardiovascular [2] and immune [9,12,13] disorders has been shown in a series of experimental and clinical investigations. A positive effect is noted in inflammatory processes of different localization [3,7]. Beneficial shifts in the biological and immunological blood indexes ensue from the use of

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