of tissue hypoxia. In addition to their direct impact on the microvessels, the ongoing action of these factors promotes plasma penetration in the basal membrane and provokes proliferation of the vascular endothelium by raising the permeability of the capillary walls for diverse types of macromolecules, this inevitably leading to occlusion of the vessel lumen and to exacerbation of the primary disturbances. Thus, the chain of pathological reactions is closed, and a vicious circle is formed. Under such conditions, correction of metabolic disturbances alone is unable to break this state, since the chain is closed by the damage to the membrane structures, and these disturbances may also aggravate each other in the absence of metabolic disorders.

Hence, diabetic microangiopathies are to be regarded as a manifestation of diabetes mellitus of a polypathogenetic nature.

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Antiepileptic Effects of Glutapyrone, a Novel Derivative of 1,4-Dihydropyridine, Administered in Combination with Sodium Valproate and Phenobarbital

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The leading trend in modern pharmacotherapy is a combined administration of drugs, which makes it possible to use the potentiation phenomenon and to act simultaneously upon a number of elements of the pathological process [1,5-9]. Although glutapyrone is a derivative of the 1,4-dihydropyridines (DHP), in contrast to classical 1,4-DHP, it has previously been shown not to affect the $^{45}Ca^{2+}$ uptake by rat brain synaptosomes or the hemodynamics [2,11]. At the same time, the profiles of anticonvulsive activity of

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TABLE 1. Effect of Glutapyrone, Sodium Valproate, and Their Combination on Generalized EpA ($M \pm m$)

Group of animals	L 1, sec	Tonic phase		Clonic phase		Time of death, sec	Number of dying animals	Severity of seizures, points
		L 2, sec	duration, sec	L 3, sec	duration, sec			
Physiological saline + corazole ($n=16$)	42.4±1.9	55.3±2.8	22.3±3.7	106.8±9.9	20.9±0.7	391.7±87.2	8	3.00±0.21
Glutapyrone + corazole ($n=16$)	47.4±2.7	68.5±2.1	23.0±1.9	116.3±17.6	23.5±3.1	675.4±177.8	8	2.62±0.21
Sodium valproate + corazole ($n=16$)	50.6±3.3 $p<0.05$	66.1±1.9	20.2±2.1	270.7±42.3 $p<0.001$	27.7±0.9	492.5±154.1	4	2.25±0.21 $p<0.02$
Glutapyrone + sodium valproate + corazole ($n=16$)	53.3±2.8 $p<0.01$	60.7±1.7	23.2±1.1	–	– $p<0.001$	–	–	1.00±0.00

Note. L 1, L 2, and L 3: latencies of first seizure manifestations and of clonic and tonic phases of generalized seizures.

glutapyrone and classical 1,4-DHP are the same [3,4,10].

The present paper focuses on the effect of glutapyrone in combination with sodium valproate and phenobarbital on generalized corazole-induced and on focal 4-aminopyridine-induced epileptic activity (EpA), as well as on electroshock-induced seizures.

MATERIALS AND METHODS

The experiments were carried out on 152 male Wistar rats weighing 220-250 g and 54 male Icr:Icl mice weighing 19-24 g. Generalized EpA was provoked in the rats by intraperitoneal injections of corazole in a dose of 75 mg/kg. The effects observed were visually recorded over 60 min. The latency of the first seizure manifestations and the latency and duration of the clonic and tonic phases (falling of the animal on its side) of the generalized seizure response and their duration, the severity of seizures, the longevity, and the mortality were determined. The severity of seizures was rated 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. The preparations were dissolved in physiological saline and administered intraperitoneally: glutapyrone in a dose of 8 mg/kg, 1 hour prior to corazole injection, and sodium valproate in a dose of 80 mg/kg, 10 min prior to corazole injection.

Focal EpA was simulated in the rats as described previously [3], the dura mater being preserved intact. The focus of EpA was created by applying filter paper moistened with a 0.017% solution of 4-aminopyridine (4-AP). Electrograms were recorded on an EEG 8S electroencephalograph (Hungary) in freely moving animals without anesthesia. The preparations were administered intraperitoneally: glutapyrone in a dose of 30 mg/kg, 1 h before EpA was provoked; and sodium valproate in doses of 80 and 150 mg/kg, 10 min prior to EpA. The amplitude-frequency characteristics, the latency of the first ictal discharge (ID), the mean and total duration of ID over the life span of the focus, and the life-span of the EpA foci were determined. In view of the possible influence of various factors, the effects of the preparations were tested in parallel with the effect of convulsant alone. For this purpose, the control animals were administered physiological saline under the same experimental conditions.

Maximal electroshock was performed with an alternating current (50 mA, frequency 50 cps, duration 0.2 sec). The preparations were injected intraperitoneally: glutapyrone in doses of 1.5 and 5 mg/kg and sodium valproate in doses of 5 and 50 mg/kg 1 and 3 h prior to electroshock; and phenobarbital 30 min prior to electroshock.

The reliability of differences between the samples was assessed using the Student t test.

RESULTS

Administration of corazole caused generalized seizures in the control rats. In some animals (7 out of 16 rats) corazole did not cause tonic-clonic seizures with falling on the side; in these animals repeated clonic seizures were observed. The mean severity of seizures in this control group was 3.00± ±0.21 points (Table 1).

Administration of glutapyrone in a dose of 8 mg/kg had no effect on the EpA (Table 1). The

TABLE 2. Effect of Glutapyrone, Sodium Valproate, and Their Combination on Aminopyridine–Induced EpA in the Rat Cerebral Cortex ($M \pm m$)

Series	Group of animals	Number of animals	L, min	Number of ID over life-span of focus	Mean duration of ID, min	Total duration of ID over life-span of focus, min	Life-span of focus, min
1	Control	15	3.36±0.54	9.40±0.82	0.75±0.08	7.33±1.65	38.47±3.81
	Experimental: sodium valproate, 150 mg/kg	9	7.22±1.68*	4.55±0.78****	0.78±0.10	3.52±0.39*	18.56±3.13****
2	Control	6	2.75±0.55	9.66±2.09	0.86±0.07	7.39±1.37	25.67±3.07
	Experimental: sodium valproate, 80 mg/kg	8	4.90±1.02	6.00±1.45	0.56±0.07	3.47±0.99*	19.9±4.52
3	Control	16	1.79±0.29	7.00±0.11	0.70±0.11	5.88±0.61	24.17±1.61
	Experimental: glutapyrone, 30 mg/kg	16	3.40±0.86	7.70±1.54	0.79±0.08	6.38±1.50	19.83±2.79
		8	3.50±0.98	3.80±0.62*	0.77±0.08	2.85±0.70***	14.08±2.92***
4	Control	9	1.86±0.51	8.82±0.77	0.49±0.08	4.89±1.13	25.78±2.51
	Experimental: sodium valproate + glutapyrone	8	5.56±1.17**	4.00±0.89****	0.31±0.05	1.78±0.60*	14.39±1.58***

Note. L: latency, time from moment of application to first ID. One, two, three, and four asterisks indicate reliability of differences for $p<0.05$, $p<0.02$, $p<0.01$, and $p<0.001$, respectively, vs. the same parameter in the control group.

mean severity of seizures in this group of animals was 2.62±0.21 points.

Sodium valproate in a dose of 80 mg/kg exhibited an antiepileptic effect which manifested itself as an increased latency of the first seizures, as well as a delay of the tonic phase of the generalized seizures (Table 1). The mean severity of seizures in the animals of this group was 2.25±0.21 points, this being lower ($p<0.02$) than the same index in the control group.

A more pronounced inhibition of the generalized EpA was observed for joint administration of glutapyrone and sodium valproate as compared to the administration of valproate alone: in none of the animals did corazole provoke either tonic-clonic or repeated clonic seizures (Table 1). The mean severity of seizures was 1.00±0.00 point in the animals of this group, which was lower ($p<0.001$) than that in the control group and in the group given just valproate ($p<0.001$).

Thus, when administered separately, glutapyrone in a dose of 8 mg/kg failed to have any effect on the generalized EpA. When administered in combination, glutapyrone in the same dose markedly potentiated the effect of valproate.

Application of 4-AP on the sensorimotor zone of the rat cerebral cortex provoked EpA: after 1-3 min, a convulsive ID (a series of high-frequency hypersynchronized discharges) appeared; the frequency of generation and the duration of ID then gradually increased, as well as the amplitude of individual discharges in the series. The life-span of the epileptic focus (from the moment of the first ID to the completion of the last one) was 40-50 min, on the average.

A marked inhibition of EpA in the focus was observed for preliminary administration of valproate in a dose of 150 mg/kg. This manifested itself as an increase of the latency of the first ID, a reduction of the frequency of generation and of the total duration of ID during the life-span of the focus, and a reduction of the life-span of the foci (Table 2, series 1). Lowering the dose of valproate to 80 mg/kg caused a decrease in the mean and total duration of ID over the life-span of the focus (Table 2, series 2). Thus, valproate in the given dose elicited a less pronounced effect as compared to the dose of 150 mg/kg.

Preliminary administration of glutapyrone in a dose of 30 mg/kg inhibited EpA in 50% of the animals (Table 2, series 3). In these animals the frequency of generation and the total duration of ID and the life-span of the foci were reliably lower than the corresponding parameters in the control group.

When glutapyrone (30 mg/kg) was used in combination with valproate (80 mg/kg), a more pronounced suppression of EpA was observed than with separate administration of these preparations in the same doses: the latency of the first ID

increased, the frequency of generation of ID was more markedly reduced, and the total duration of ID and the life-span of the foci were shortened (Table 2, series 4). This effect of combined administration may be compared to that of separate administration of these preparations in high doses: of valproate in a dose of 150 mg/kg or of glutapyrone in a dose of 80 mg/kg.

Glutapyrone and sodium valproate in doses of 1-100 mg/kg exerted no effect on electroshock-induced seizures in mice. Glutapyrone in a dose of 5 mg/kg, administered 1 and 3 h prior to electroshock, markedly (by 49.9 and 42.3%, respectively; $p<0.05$) enhanced the antiepileptic activity of phenobarbital: ED_{50} of phenobarbital was 35.5 mg/kg, whereas against the background of glutapyrone it was 17.8 and 20.5 mg/kg ($p<0.05$). Sodium valproate under similar experimental conditions had no effect on the anticonvulsive activity of phenobarbital.

The results of our study attest to the advisability of administering glutapyrone in combination with valproate and phenobarbital, since this results in the potentiation of the effect of each preparation for much lower doses. This reduces the likelihood of adverse effects, which is especially important in the case of prolonged treatment.

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Restoration of the Mast Cell Population in Rat Mesentery

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There is no consensus on the genesis and role of the mast cells (MC) of the connective tissue in the normal life of the human and animal organism. Much more information is available in reports dedicated to the role of MC in pathological processes. The theory of a "local adaptation syndrome" [7] with a leading role being played by connective-tissue MC, which were considered to be unicellular glands, arose in the 70s. According to the Selye theory, the biologically active substances secreted by MC provide for the local adaptation of tissue to stress effects, just as glucocorticoids of the

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