SEIZURES INDUCED BY KYNURENINE AND QUINOLINIC ACID AS A SENSITIVE TEST TO EVALUATE THE ANTICONVULSANT ACTIVITY OF GABA-ERGIC DRUGS

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The writers previously showed that some GABA derivatives have anticonvulsant activity against seizures induced by kynurenine (K) and quinolinic acid (QA), the most powerful convulsant metabolites of the amino acid tryptophan, and probable participants in epileptogenesis [3]. Fenibut (β-phenyl-γ-aminobutyric acid), sodium hydroxybutyrate (GHBA), and phepyron (4-phenylpyrrolidone-2), with no anticonvulsant action on known models of generalized seizures [1, 7, 8], exhibited their activity [6].

It was decided to continue the study of GABA-ergic drugs, including those with different types of GABA-ergic mechanisms: the chlorine derivative of fenibut, baclofen (lioresal, β-4-cholorophenyl-GABA), whose complex mechanism of action incudes excitation of GABA receptors [11] and inhibition of glutamatergic transmission [16], muscimol, an agonist of GABA receptors, the GABA-transaminase inhibitors aminohydroxyacetic acid (AHAA), and depakin (N-dipropyl acetate) [5, 13, 19], and also diazepam and phenobarbitol, whose action is linked with the benzodiazepine–GABA–chlorine–ionoforms receptor complex [15].

To discover the particular features of the anticonvulsant action of the compounds and to compare them with clinical data it was decided to compare effectively GABA-ergic drugs on different models of seizures.

Considering the recently published report [4] that intraperitoneal injections of drugs may have an anticonvulsant action on account of the "nonspecific peritoneal syndrome," tests were also carried out to determine whether the anticonvulsant activity of fenibut and GHBA is still present if it is given by the peroral route.

EXPERIMENTAL METHOD

Experiments were carried out on 1080 male SHR albino mice weighing 14-20 g, from the "Rappolovo" nursery, during the winter and spring. Fenibut, GHBA, baclofen, and AHAA were dissolved in distilled water, whereas depakin (calcium salt) was used in the form of an aqueous emulsion in Tween-85. Before and after administration of the drugs the animals (10 mice in each group) were kept in metal boxes measuring 20 × 15 × 10 cm. After 30 min, 50 μg of L-kynurenine sulfate (1% solution, from Sigma, USA) or 5 μg QA (1% solution from Sigma) was injected by means of a semiautomatic apparatus [18] into the cerebral ventricles (through the right lateral ventricle). For comparison typical convulsants were used: strychnine sulfate (0.01% aqueous solution) or metrazol (0.8% aqueous solution), which was injected subcutaneously. The parameters of maximal electric shock (MES) were as follows: 50 mA, 0.2 sec; steel oval electrodes. All convulsants were used 30 min after intraperitoneal and 60 min after peroral administration of the drugs. The effects were recorded visually for 10 min after intraventricular injection and MES, and for 30 min after subcutaneous injection of the convulsants. The latent period of the seizures was measured by a stopwatch.

The following four principal parameters were determined: latent period of onset of seizures, frequency of clonic convulsions in the group, frequency of tonic extensions in the group, mortality. The significance of differences between latent periods was estimated by Student's t test, that of the other parameters by the chi-square test.

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TABLE 1. Comparison of Effectiveness of GABA-ergic Drugs on Various Models of Seizures

Preparation tested	Mode of administration	L-kynurenine sulfate, intraventricularly, 50 μg	Quinolinic acid, intraventricularly, 5 μg	Strychnine sulfate, subcutaneously, 1 mg/kg	Metrazol, subcutaneously, 80 mg/kg	MES, 50 mA, 0.2 sec
GABA	Intraventricularly	P 1—5	P 1—5	1—5	P 1—5	0 1—5
Muscimol	Intraventricularly	Pel 0,05	P 0,05	CE 0,05	P 0,05	—
	Intraperitoneally	P 1	0 1		—	—
Fenibut	Perorally	P1 E Pel 400 800 1200	p P pcel 400 800 1200	—	0 1200	1+ 200—400
Baclofen	Intraperitoneally	p Pc 5 20	0 p el 5 10 20	P1 20	0 20	1 el 10 20
GHBA	Perorally	P1 P Pcel 400 800 1200	P1 P Pcel 400 800 1200	—	0 1200	1+ 200—400
Depakin	Intraperitoneally	P cel 300 450	Pel T 300 450	P1 Pel 300 450	Pcel 300	el cel 300 450
AHAA	Intraperitoneally	P 10	p 10	p 10	P.el 10	0 10
Phenobarbital	Perorally	Pel 160	Pel 160	—	T 160	1+ el+ T+ 20 40 80
Diazepam	Intraperitoneally	p p T 15 25 37,5	Pel el T 7,5 15 37,5	Pcel 2,5—15	Pcel T 0,5 1	0 1 el T 1 2,5 5—7,5 15

Legend. p) Lengthening of latent period of seizures ($P < 0.05$), p) $P < 0.02$; P) $P < 0.01$; P) $P < 0.001$; P.) shortening of latent period; c) significant decrease in number of animals in group with clonic convulsions; e) significant decrease in number of animals in group with clonic extensions; l) decrease in mortality; T) complete prevention of seizures; 0) absence of effect.

EXPERIMENTAL RESULTS

When given by the peroral route, fenibut and GHBA preserved their anticonvulsant effect against seizures induced by K and QA. The effective doses were 2-6 times higher than the doses described previously for intraperitoneal injection [6].

In doses of 1200 mg/kg fenibut and GHBA were effective with respect to all seizures, reduced the frequency of clonic convulsions and of tonic extensions in the group, and lowered mortality. In the same dose they had no effect on metrazol seizures. Consequently, the anticonvulsant activity of fenibut and GHBA was not due to the "nonspecfiic peritoneal syndrome." The weak anticonvulsant activity of muscimol and GABA, exhibited on all models of seizures except those induced by strychnine, purely by lengthening of the latent period, agreed completely with data in the literature [14, 20].

Phenobarbital, when given perorally (160 mg/kg), proved to be more effective in seizures induced by metrazol, and inhibited all manifestations of its convulsant action. In seizures induced by K and QA, on the other hand, phenobarbital did not change the frequency of clonic convulsions, i.e., it was less effective than in seizures induced by metrazol. This comparison confirms the earlier conclusion that GABA derivatives are more effective in seizure states induced by K and QA [6], and also the important role of inhibition of the GABA system in the genesis of seizures induced by kynurenine [2].

Baclofen was most effective against seizures induced by K and QA. It did not affect seizures induced by metrazol, in agreement with other workers' findings [10].

Neither depakin nor AHAA were found to have selective anticonvulsant activity against seizures induced by K and QA, which might have been expected considering the GABA-postive character of their action. This was probably due to the multiplicity of effects of depakin and AHAA [17]and also to the fact that a definite role in the mechanism of their action is played, not by GABA-positive, but by other influences. The two drugs were most effective against seizures induced by metrazol, confirming observations by other workers [12].

The sharp difference between baclofen, on the one hand, and depakin with AHAA, on the other hand, in the metrazol test points to differences in the mechanisms of their action, although all are GABA-positive drugs. Selective anticonvulsant activity in metrazol seizures reflects the similarity of depakin and AHAA to tranquilizers of the benzodiazepine series, in agreement with data in the literature [9]. The extremely low effectiveness of diazepam against seizures induced by K and QA has been noted.

The kynurenine test proved to be highly sensitive to several drugs. For instance, fenibut, GHBA, and baclofen exhibited marked anticonvulsant action against kynurenine seizures, but had only very weak action against seizures induced by other substances.

The results suggest that seizure states induced by kynurenine (K and QA) are currently the most sensitive model for the experimental study of the anticonvulsant activity of known and new GABA-ergic drugs of the fenibut, baclofen, and GHBA type.

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STIMULUS-DEPENDENT Na-CHANNEL BLOCKADE IN ISOLATED RAT MYOCARDIAL CELLS BY THE ANTIARRHYTHMIC N-PROPYLAJMALINE (NEOGILURITMAL)

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It was shown previously that blockade of Na currents in nerve fibers by amine local anesthetics [6, 7, 9] and antiarrhythmics [1, 16, 12] can be reversibly potentiated by rhythmic depolarization of the membrane. Analysis of the mechanism of this stimulus-induced cumulative block led to a number of important conclusions regarding the molecular organization of Na channels in the nerve fiber membrane [4]. However, the question of the applicability of these ideas to Na channels of myocardial cells has not been completely settled, because pharmacological analysis of the properties of myocardial Na channels is still only in the initial stages of its development.

In the investigation described below the action of an antiarrhythmic — a quaternary derivative of ajmaline, namely N-propylajmaline (NPA) — on Na currents through a microregion of the membrane (the patch clamp method) of an enzymically isolated rat myocardial cell was studied.

EXPERIMENTAL METHOD

Cells were isolated by the method described in [10]. Full details of the experimental procedure were described previously [2]. The cell selected under the microscope was transferred to a working chamber containing a solution of the following composition (in mM): NaCl 130, KCl 5.4, $MgSO_4$ 1.2, $CaCl_2$ 0.9, glucose 11, MOPS-buffer 20 (pH 7.4). The experiments were carried out at room temperature (20-22°C). By means of a V-shaped polyethylene sucker, with a pore 5-7 μ in diameter, electrodes were connected to a small area of the cell membrane. The control solution in the sucker had the composition indicated above, with the addition of 1 mM $MnCl_2$ and 1 mM 4-aminopyridine to block Ca- and K-channels, respectively. The solutions in the tip of the sucker were changed approximately in the course of 1 sec.

The currents were measured by a "virtual ground" circuit, followed by filtration down to the 3 kHz band. In all experiments the potential was assigned from the level of the cell resting potential (V = 0 mV).

EXPERIMENTAL RESULTS

Replacement of the control solution in the sucker by solution containing 1×10^{-5} to 2×10^{-5} M NPA caused no appreciable changes in amplitude of the Na current (I_{Na}) during the first 2-3 min, provided that the membrane was not stimulated during this time. In conjunction with rhythmic depolarization of the membrane, however, NPA caused progressive decline in I_{Na}.

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