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EFFECT OF ANTICONVULSANTS ON SEIZURES INDUCED BY KYNURENINE, QUINOLINIC ACID, STRYCHNINE, AND METRAZOL

I. V. Ryzhov and I. P. Lapin

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KEY WORDS: kynurenine; quinolinic acid; anticonvulsants; seizures.

Experimental treatment of seizures induced by kynurenine (K) and quinolinic acid (QA), the most active convulsant metabolite of tryptophan, is particularly interesting because this model differs from others [6, 7, 11] in the fact that it is produced with the aid of endogenous brain metabolites. Consequently, if the efficacy of anticonvulsants on models and against various types of seizures in man is compared, a closer understanding can be obtained of seizures in whose genesis a role is played by K and QA. It was found previously that kynurenine-induced seizures are selectively weakened by the inhibitory amino acids taurine and glycine [8], whereas seizures induced by QA are weakened by GABA [9] and in agonist muscimol [8], which are also effective against K.

EXPERIMENTAL METHOD

Experiments were carried out on 1040 male SHR albino mice weighing 18-25 g in the fall. The anticonvulsants were dissolved in distilled water [all in powder form except diazepam (Seduxen solution in ampules); aqueous emulsions of primidone (hexamidine) and benzobarbital (benzonal) were prepared in 1% tragacanth solution] and were injected intraperitoneally. Animals of the control groups, which accompanied each experiment, received injections of distilled water. Before and after injection of the anticonvulsants the animals (10 mice in each group) were kept in metal boxes measuring 20 × 15 × 10 cm. By means of a semiautomatic apparatus [12] 50 μg of DL-K-sulfate (from Sigma, USA; 1% solution) or 5 μg QA (from Sigma, USA; 0.1 % solution) was injected 30 min later into the cerebral ventricles (through the right lateral ventricle). Two typical convulsants were used for comparison: strychnine sulfate (0.01% aqueous solution) and metrazol (0.8% aqueous solution), and these were injected subcutaneously as aqueous solutions 30 min after the anticonvulsants. All effects were recorded visually for 10 min after intraventricular and 30 min after subcutaneous injection of the convulsants. Four main parameters were determined: 1) the latent period of onset of seizures, 2) the frequency of clonic seizures in the group, 3) the frequency of tonic extensions in the group, 4) mortality.

The significance of differences in latent period was evaluated by Student's t test, and of the other parameters by the chi-square test.

EXPERIMENTAL RESULTS

The doses of convulsants (K, QA, strychnine, and metrazol) used were about equally effective (Table 1) as regards the frequency of clonic convulsions in the group (96, 95, 96, and 97% of animals respectively), although they differed in the frequency of tonic extension (14, 48, 89, and 60% of animals respectively) and in mortality (36, 65, 80, and 52%).

All the anticonvulsants tested had moderate anticonvulsant activity against seizures induced by K, which was manifested only as lengthening of the latent period of the seizures. Strengthening of this effect with an increase in dose was observed only in the case of primidone. Of all the drugs only benzobarbital reduced the frequency of clonic convulsions.

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TABLE 1. Comparison of Sensitivity of Seizures Induced by K, QA, Strychnine, and Metrazol to Anticonvulsants

Anticonvulsants	DL-K	QA	Strychnine (1 mg/kg, subcutaneously)	Metrazol (80 mg/kg, subcutaneously)
Phenobarbital	LLL 5 10 40	Le em L 5 10 40	O O 10 40	Lem Lcem 10 20 and 40
	l	Lem em Lcem	O L L	O
Phenytoin	10 40 O L L *l*	10 20 40 L Lm cm	10 20 40 O O	40 0
Primidone	5 10 25 50 L L Lc	10 25 50 L Lm Lm	25 50 O L * O	50 O
Benzobarbital	10 25 50	10 25 50	10 25 50	50
Troxidone	L 200	L 200	Lcem T 200 300	Lcem T 200 300
Diazepam	*l l* T 15 25 37,5	Lem em T 7,5 15 37,5	L cem 7,5 15	Lcem T 0,5 1

Legend. 1) Lengthening of latent period of convulsions ($P < 0.05$); 1) $P < 0.02$; L) $P < 0.01$;) $P < 0.001$; L*) shortening of latent period; c) significant decrease in number of animals with clonic convulsions; e) significant decrease in number of animals in group with tonic extension; m) decrease in mortality; T) complete prevention of convulsions; 0) no effect; numbers below letters indicate doses of anticonvulsants (in mg/kg).

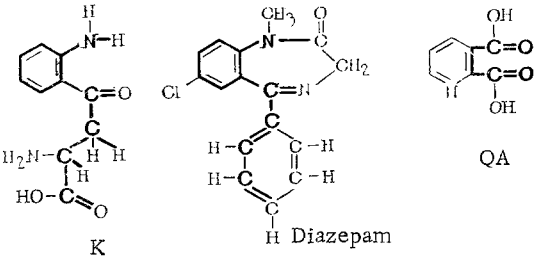

Fig. 1. Chemical structure of K, diazepam, and QA.

Diazepam, which was highly effective in a dose as low as 0.5 mg/kg against metrazol convulsions, moderately lengthened the latent period of kynurenine convulsions only in doses of 15 and 25 mg/kg. Complete prevention of kynurenine convulsions was observed with a dose of 37.5 mg/kg. Activity of the diazepam preparation in antimetrazol and antistrychnine tests agreed completely with data in the literature [1, 2]. ED_{50} for diazepam against electric shock was 23 mg/kg [2], but against audiogenic seizures it was 0.5 mg/kg [10]. The exceptionally low efficacy of diazepam (even in doses 50 times greater than ED_{50} against metrazol and 1.5-2 times greater than ED_{50} against strychnine) was unique, for no information could be found in the literature on a convulsant against which diazepam was practically ineffective. Diazepam also has a very weak effect on electrical convulsions [2]. The similarity of the chemical formulas of K and diazepam (Fig. 1) suggests that K as an endogenous metabolite may be a more powerful competitive antagonist of diazepam for the benzodiazepine receptor. Although the carbon chain of the K molecule does not have a rigid configuration because of the absence of a double bond (which is present in the phenol ring of diazepam), the possibility cannot be ruled out that this configuration may have such an arrangement, among all possible arrangements, both during interaction with the receptor or with a certain endogenous substance and after its probable metabolic conversions: deamination followed by dehydration.

The anticonvulsants tested had a much stronger action on QA-induced convulsions than on kynurenine convulsions. All the preparations except troxidone reduced mortality and the frequency of tonic extension (phenobarbital, phenytoin, diazepam) and clonic convulsions (primidone). Diazepam reduced the frequency of tonic extension and the mortality after QA but had no effect on clonic convulsions even in doses of 7.5 and 15 mg/kg, which are effective against strychnine. In a dose of 37.5 mg/kg diazepam prevented clonic convulsions also. The convulsant effect of QA and K may be connected with the common fragment in their structure, namely the two carbonyl groups separated by two carbon atoms. Of all the kynurenines, this fragment is present in QA and K, the most potent endogenous convulsants. It is also present in aspartic acid, which has an excitatory and convulsant action. The higher convulsant activity of QA than of K and aspartic acid may be connected with the originally fixed distance between the C=O groups in the QA ring. This distance is variable in the mobile side chain of K and aspartic acid. Qualitative differences in the effects of K and QA may also be connected with differences in their chemical structure and, in particular, with the residue of the indole ring and the α-amino acid side chain of K.

Only troxidone and diazapam had a clear effect on strychnine convulsions, and inhibited all manifestations of convulsant action. Phenytoin simply lengthened the latent period of the seizures. Comparison of the effective doses shows that troxidone was much less active than diazepam; moreover, it selectively affected metrazol and strychnine convulsions, whereas diazepam was active against all three convulsant seizures, and most active against metrazol.

Diazepam, troxidone, and phenobarbital had a clear anticonvulsant action against metrazol convulsions, however, diazepam had an action which was many times stronger.

Primidone and benzobarbital were selectively effective against convulsions induced by K and QA. In the doses tested (10 and 50 mg/kg) they did not affect strychnine and metrazol convulsions. These doses are much lower than those effective against electric shock and metrazol [2]. Phenytoin was most effective against QA, for only in that case did it reduce the frequency of tonic extensions and the mortality.

The selective activity against K and QA of primidone, benzobarbital, and phenytoin — anticonvulsants effective in the treatment of grand mal — suggests that K and QA may be concerned in the genesis of seizures of this type. Since K and QA potentiate the activity of a penicillin focus in the hippocampus of rats and frogs [3, 4], which is regarded as a model of psychomotor seizures [5], K and QA probably participate in the genesis of psychomotor epilepsy also.

The character of the model of convulsions (predominance of major or psychomotor seizures) may depend on the method of administration of the kynurenines — whether injected into the cerebral ventricles or directly into the hippocampus (or other epileptogenic structures). Besides the method of administration, the species of animal may also be important: Differences between threshold convulsive doses of K and QA, injected into the cerebral ventricles, are considerable in mice, rats, and frogs.

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EFFECT OF ACETAZOLAMIDE ON CARBONIC ANHYDRASE ACTIVITY IN THE BLOOD AND GASTRIC MUCOSA AND ON THE PEPSINOGEN CONTENT IN THE GASTRIC MUCOSA OF RATS

P. G. Storozhuk, I. M. Bykov,
Yu. P. Malyshev, and T. N. Litvinova

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An important role in the mechanism of hydrochloric acid biosynthesis by the parietal cells of the gastric mucosa is ascribed to carbonic anhydrase [5], activation of which by pentagastrin, histamine, carbachol, theophylline, or cyclic 3,5-AMP increases hydrochloric acid secretion [6, 9-11, 13]. Liberation of HCl is stimulated by electrical stimulation of the gastric mucosa [7] and by protein kinase [12]. Acetazolamide and atropine abolish the activating action of pentagastrin and carbachol on carbonic anhydrase [10].

However, there is nothing in the literature on the subject of dependence of the level of pepsin secretion on carbonic anhydrase activity.

The object of this investigation was to study the effect of acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide) on carbonic anhydrase activity in the blood and gastric mucosa and on the pepsinogen content in the gastric mucosa.

EXPERIMENTAL METHOD

Experiments were carried out on 70 male albino rats weighing 120-150 g. Blood for testing was taken from a cervical vein. To obtain a homogenate of the mucosa the stomach was opened, washed with cold distilled water, dried with filter paper, and the mucosa was separated, and a weighed sample of the mucosa was homogenized in water (ratio 1:100). Carbonic anhydrase activity in the blood and homogenate was determined by a micromethod [4]. The pepsinogen concentration in the gastric mucosa was studied after its conversion into the active form — pepsin. For this purpose the homogenate was treated with 0.1 N HCl (ratio 10:1)

TABLE 1. Carbonic Anhydrase Activity in Blood (in units/μl) and Gastric Mucosa (in units/mg tissue) and Pepsin Activity in Gastric Mucosa (in units/g tissue) after a Single Dose of Milk, Histamine, and Acetazolamide in Various Combinations (M ± m)

Group of animals	Stimulus of gastric secretion	Carbonic anhydrase		Pepsin in gastric mucosa
		in blood	in gastric mucosa	
1 (n=10)	Fasting state	67,6±9,42	4,22±0,46	34,9±2,72
2 (n=10)	Milk	88,2±12,58 (+33,4%)	5,02±0,43 (+19%)	42,4±3,25 (+21%)
P_{1-2}		<0,01	<0,01	<0,01
3 (n=10)	Histamine	105,5±8,94 (+55,8%)	6,47±0,61 (+53%)	48,5±3,81 (+39%)
P_{1-3}		<0,01	<0,01	<0,01
4 (n=10)	Acetazolamide + milk	36,1±5,47 (—59%)	3,21±0,45 (—36%)	33,3±1,77 (—21,5%)
P_{2-4}		<0,01	<0,01	<0,01
5 (n=10)	Acetazolamide + histamine	36,6±4,81 (—65%)	3,09±0,50 (—52%)	39,0±2,98 (—19,6%)
P_{3-5}		<0,01	<0,01	<0,01

Department of Biochemistry, Red Army Kuban Medical Institute, Krasnodar. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 699-701, December, 1981. Original article submitted February 11, 1981.

