COMPARISON OF ANTICONVULSANT AND ANTIARRHYTHMIC EFFECTS OF THE BENZODIAZEPINE RECEPTOR AGONIST PHENAZEPAM

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Functional insufficiency of the GABA-ergic system plays an important role in the pathogenesis of epilepsy and of seizure activity. The GABA level, activity of the enzyme of GABA synthesis, glutamate decarboxylase, and binding of ^{3}H-GABA in the brain are depressed in epileptics [6, 15]; inhibition of GABA synthesis and blockade of GABA-receptors lead correspondingly to increased seizure activity [17]. It has been shown in recent years that blockade of GABA receptors by bicuculline [11] in doses not causing convulsions provokes cardiac arrhythmias. These observations are in agreement with the well known fact that the GABA-ergic system of the brain has a tonic inhibitory action on the centers of adrenergic control of the heart [9], excessive excitation of which causes arrhythmias associated with stress and myocardial ischemia [16, 18]. Data on the role of disturbance of function of the GABA-ergic system in the pathogenesis of both seizure activity and cardiac arrhythmias are of essential practical importance. In recent years, for instance, it has been shown that certain activators of the GABA-ergic system, such as sodium valproate, possess effective anticonvulsant action [8, 19] and, at the same time, they are antiarrhythmics [5].

Agonists of benzodiazepine receptors, interacting with a supramolecular complex containing GABA-receptor, benzodiazepine receptor, and chloride ionophore, allosterically bound together, potentiate the many different effects of the GABA system [12, 13]. It can accordingly be postulated that these compounds may also possess anticonvulsant and antiarrhythmic activity simultaneously.

The aim of this investigation was to compare the anticonvulsant and antiarrhythmic action of phenazepam [2], an agonist of benzodiazepine receptors, used clinically as a tranquilizer [7].

EXPERIMENTAL METHOD

The anticonvulsant effect of phenazepam was studied on noninbred male albino mice weighing 20-24 g, using traditional methods [1]. Drugs affecting individual components of the above-mentioned GABA-benzodiazepine-receptor complex were used as factors inducing seizures: bicuculline — a specific antagonist of $GABA_A$-receptors, metrazol, a GABA antagonist, picrotoxin, a blocker of the chloride ionophore part of the complex, Ro 5-3663, an inhibitor of binding of α-dihydropicrotoxin, affecting interaction of GABA and the benzodiazepine subunit of the complex, and thiosemicarbazide, altering the GABA content. Two other factors also were used: maximal electric shock and strychnine, involving the glycinergic system in the realization of seizures. Maximal electric shock was produced by electrical stimulation with parameters of 50 Hz, 50 mA, and duration 0.2 sec. The electrodes were located intracorneally. Statistical analysis was carried out with calculation of values of ED_{95} and ED_{50} by probit analysis [14]. ED_{95} is the effective dose of the substance inducing seizures in 95% of animals, ED_{50} the effective dose of phenazepam abolishing seizures in 50% of animals. Phenazepam was injected intraperitoneally 40 min before injection of the convulsant.

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TABLE 1. Effect of Phenazepam on Convulsant Effects of Various Analyzers of GABA-Benzodiazepine Receptor Complex in Mice

Factor inducing seizures	ED_{95}, mg/kg	Mode of administration of substances	Anticonvulsant activity of phenazepam, ED_{50}, mg/kg
Metrzol	140,0	Subcutaneously	0,08 (0,058—0,112)
Bicuculline	3,0	»	0,5 (0,38—0,65)
Thiosemicarbazide	28,0	Intraperitoneally	0,1 (0,08—0,12)
Picrotoxin	16,0	Subcutaneously	0,6 (0,5- -0,72)
Ro 5—3663	34,0	»	0,64 (0,5—0,72)
Strychnine	1,7	»	26,1 (20,8—33,8)
Maximal electric shock	—	—	8,4 (7,0—10,8)

The antiarrhythmic effect of phenazepam was studied on male Wistar rats weighing 230-300 g, during acute ischemia and reperfusion of the heart in the intact animal and on the isolated heart. Experiments on the whole animal were conducted on two groups of rats: one group received phenazepam, the other was the control.

Experiments were carried out under pentobarbital anesthesia (50 mg/kg) with open chest and artificial ventilation of the lungs. The electrocardiogram (ECG) was recorded in standard lead II on a "Mingograf-34" apparatus (Siemens—Elema, West Germany — Sweden). Against the background of ECG recording acute ischemia was produced by ligation of the left coronary artery for 10 min, which was followed by reperfusion of the myocardium for 10 min by removal of the ligature. Under these circumstances the heart rate and the abundance of three types of arrhythmias — extrasystoles, ventricular tachycardia, and ventricular fibrillation — were determined.

Three parameters were calculated for each type of arrhythmia: the frequency of onset of each type of arrhythmia (the number of animals in the group developing that arrhythmia); the total duration of arrhythmia of that particular type in the group; the average duration of arrhythmia of that type calculated per animal; all groups, i.e., those with and without arrhythmias, were taken into consideration. These parameters also were calculated in total for all types of arrhythmias. Experiments with myocardial ischemia and reperfusion on the isolated heart were carried out by perfusing the heart by Langendorf's method with Krebs—Henseleit solution (pH 7.3-7.4; 95% O_2 + 5% CO_2; 36.5 ± 0.5°C), with recording of the mechanogram and ECG by means of specialized modules of the RM-6000 polygraph and VC-9 oscilloscope ("Nihon Kohden," Japan). Ischemia and reperfusion were carried out by tightening and loosening the ligature on the left coronary artery. The duration of ischemia was 20 min and of reperfusion 5 min. The same parameters of the arrhythmia were determined as in experiments on the whole animal. The numerical results for the duration of the arrhythmias were subjected to statistical analysis by the Wilcoxon—Mann—Whitney U test and the significance of differences in the frequency of onset of arrhythmias was determined by the chi-square test. In experiments on the whole animal phenazepam was injected intraperitoneally in a single dose of 1 mg/kg 1 h before creation of ischemia. The dose was chosen in preliminary experiments as being optimal relative to its antiarrhythmic effect within the range of 0.1-1.5 mg/kg.

The control animals received physiological saline. In experiments on the isolated heart phenazepam was added to the perfusion solution 10 min before ischemia, and its administration continued throughout the experiment in concentrations of 6-60 ng/ml, corresponding to the doses used on the whole animal.

EXPERIMENTAL RESULTS

Assessment of the anticonvulsant effect of phenazepam showed that it possesses high activity as an antagonist to bicuculline, metrazol, picrotoxin, Ro 5-3663, and thiosemicarbazide, but is ineffective against convulsions evoked by electric shock and strychnine (Table 1). The results are evidence that phenazepam has a marked effect mainly on seizures induced by insufficiency of various components of the GABA-ergic system: the GABA receptor, coupling of the GABA- and benzodiazepine receptors, the chloride channel, and the GABA level, but does not modify seizures induced by other factors (maximal electric shock, strychnine), which involve other neurotransmitter systems. Thus the dominant place in the anticonvulsant effect of phenazepam is occupied by its potentiating action on the GABA-ergic system.

Table 2 gives the results of investigation of the effect of preliminary administration of phenazepam on the development of ischemic and reperfusion induced cardiac arrhythmias in the whole animal. In animals receiving phenazepam, the total duration of all types of cardiac arrhythmias during ischemia and reperfusion was significantly less than in the control animals. For instance, the value of this parameter, calculated per animal, during ischemia was 38.9 ± 5.6 sec in the group receiving phenazepam, i.e., 2.3 times less than in the control ($p < 0.01$), compared with 16.2 ± 4.2 sec during reperfusion, i.e., 4.7 times less than in the control

TABLE 2. Effect of Phenazepam on Cardiac Arrhythmias during Ischemia and Reperfusion ($M \pm m$, $n = 12$)

Parameter	Variant of experiment			
	ischemia		reperfusion	
	control	phenazepam	control	phenazepam
Total duration of all types of arrthmias: per animal, sec	92,2±10,9	38,9±5,6*	76,7±14,2	16,2±4,2*
Extrasystoles (ES): number of animals with ES	12	12	12	10
mean duration of ES per animal, sec	32,9±4,2	23,9±3,2	6,3±2,1	4,1±1,0
Ventricular tachycardia (VT): number of animals with VT	12	10	12	11
mean duration of VT per animal, sec	42,6±8,6	13,5±2,9*	47,7±9,4	11,6±3,7*
Ventricular fibrillation (VF): number of animals with VF	8	5	7	2*
mean duration of VF per animal, sec	16,9±5,5	1,5±0,6*	22,7±15,3	0,5±0,4*
Number of animals dying	0	0	1	0

Legend. n) Number of experiments; $^*p < 0.05$.

($p < 0.001$). It is also essential to note that the severest form of arrhythmia, ventricular fibrillation, occurred less frequently in animals receiving phenazepam and lasted a shorter time. For instance, during reperfusion fibillation occurred in seven of 12 animals in the control group, but in only two of 12 animals in the group receiving phenazepam, i.e., 3.5 times less frequently ($p < 0.05$). The mean duration of fibrillation per animal in this case was 1.5 ± 0.6 sec during ischemia and 0.5 ± 0.4 sec during reperfusion, or 10 and 45 times less respectively than in the control ($p < 0.001$).

Thus in the whole animal phenazepam possesses a significant antiarrhythmic action in acute ischemia and reperfusion of the heart. In experiments on the isolated heart phenazepam had no protective, antiarrhythmic action.

On the whole the results of comparison of the anticonvulsant and antiarrhythmic action of phenazepam indicate that this benzodiazepine receptor agonist possesses high anticonvulsant activity against those types of seizures that are the result of a disturbance of function of the GABA-ergic system, and also exhibits powerful antiarrhythmic action in ischemia and reperfusion of the heart. This antiarrhythmic effect, moreover, is central in nature, for it is not realized on the isolated heart. This fact is in agreement with modern views on the modulating action of the GABA-ergic system both on motor centers [3] and on autonomic centers regulating cardiac activity [4, 9, 10]. This fact is in agreement with data on the important role of insufficiency of the GABA-ergicsystem both in the pathogenesis of epilepsy [6, 15, 17] and in the genesis of arrhythmias [4, 5]. The essential fact is that the antiarrhythmic action of phenazepam was obtained with small doses of the drug. This raises the problem of its use in clinical cardiology.

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ETHANOL- AND ACETALDEHYDE-METABOLIZING SYSTEM OF RAT LIVER DURING DEVELOPMENT OF TOLERANCE TO ETHANOL

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During single and repeated contact of the body with ethanol, the latter is metabolized by many different enzymes: alcohol dehydrogenase (ADH), aldehyde dehydrogenase (AldDH), etc. The duration of narcotic sleep induced by ethanol, used as a criterion to assess tolerance, depends on the levels of both ethanol and acetaldehyde in the body. The ratio between them is determined by the state of the metabolic systems for the two compounds [2, 5].

The aim of this investigation was to study the time course of changes in activity of ADH, AldDH, and other enzymes in the rat liver during the development of the initial stages of alcohol tolerance.

EXPERIMENTAL METHOD

Experiments were carried out on 80 male rats weighing 140-200 g, kept on a standard animal house diet. A 25% solution of ethanol was injected intraperitoneally into the animals in a dose of 3.5 g/kg body weight. The injections were given daily in the morning (10-11 a.m.) by the same persons in the same room. The time for loss of the turning reflex, namely 2-4 min after injection of ethanol, and the time for recovery of this reflex were recorded. The time spent by the rats in the side position reflected the duration of narcotic sleep. The animals were divided into four groups (20 in each group): in group 1 a single injection of ethanol was given, three injections in group 2, seven in group 3, and 10 in group 4. The rats were decapitated 24 h after the last injection of ethanol. The tissues were quickly frozen in liquid nitrogen. ADH activity with ethanol and acetaldehyde as substrates [1], AldDH activity [8], and protein [7] and DNA [3] concentrations in the liver were determined. Activity of the NADPH-dependent ethanol-metabolizing system (EMS) was estimated in liver microsomes [6].

EXPERIMENTAL RESULTS

During repeated administration of ethanol at intervals of 24 h tolerance developed to it, as shown by a sharp fall in the duration of ethanol-induced sleep. The following groups of rats were found: those not sleeping, classed as highly tolerant (HT); those sleeping under 30 min, i.e., short-sleepers (SS), and those sleeping more than 60 min, i.e., long sleepers (LS). The general reduction in the duration of sleep was accompanied by changes in the number of HT, SS, and LS rats. The data given in Fig. 1 indicate a sharp decrease in the number of LS individuals and their moving into the SS and HT categories, reflecting an increase in tolerance of the population as a whole to ethanol. There was also a decrease in the duration of sleep in the LS from 130-100

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