of the estrous cycle, the blood ethanol levels in animals coming round from narcosis were about equal but no fall in ethanol concentration was observed under these circumstances (Fig. 2).

The short duration of ethanol narcosis in the stage of estrus and its longer duration in the stage of diestrus ($t_D/t_\theta > 1$), the low endogenous ethanol level in the stage of estrus, and the rapid fall in the blood ethanol concentration after administration of a narcotic dose during estrus are thus characteristic of female rats which choose ethanol in preference to water under free choice conditions and consume it in larger doses.

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NEUROPHARMACOLOGIC PROPERTIES OF PYRACETAM DERIVATIVES

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The high clinical efficacy of pyracetam justifies the study of the mechanism of its action and the search for new compounds with neurotropic activity. The aim of the investigation described below was to study some derivatives and analogs of pyracetam, with neurotropic activity, synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR.*

EXPERIMENTAL METHOD

In stage I the compounds were tested on male mice by the basic tests of neurotropic screening: general action, motor activity, action on the effect of amphetamine, pentobarbital, ethanol, chlorpromazine, various convulsants (Table 1), and thiopental sodium. To discover potential neurotropic drugs models of oxygen insufficiency [2, 3] were used: normobaric hypoxia (length of survival in an airtight chamber) and hypobaric hypoxia (pressure chamber, experiments under two conditions: survival after 15-min exposure at an "altitude" of 11,000 m or determination of the tolerated altitude ceiling). The most active substances according to these tests were studied in electrophysiological experiments. Their effect on the recovery cycle of evoked potentials (RCEP) recorded in the somatosensory cortex during paired stimulation of the sciatic nerve, and averaged by means of an LP-4840 analyzer, was investigated in acute experiments on 20 rats (details of the method were described previously [4]). The threshold dose in which pentobarbital induces particular phases of ECoG depression in animals after preliminary injection of physiological saline (control) or of the test substance were determined in experiments on twelve rabbits. Potential neurotropic drugs were studied on

*Most of these compounds are mentioned in the patent literature [9, 10], but no data have been published on the spectrum of their pharmacologic activity.

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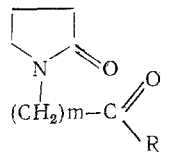
TABLE 1. Comparative Activity of Pyracetam Derivatives in Neurotropic Screening Tests[1]



Compounds			Anti-hypoxic effect[2]	Motor activity during 15 min			Seizures (mortality, percent)		Duration of anesthetic effect of thiopental sodium in a dose of 30 mg/kg	Change in background EEG[4]	Toxic doses, g/kg
code numbers	m	R		spontaneous	activated by amphetamine in dose of 5 mg/kg	inhibited by pentobarbital[3] in dose of 20 mg	bicuculline, 3 mg/kg	metrazol, 110 mg/kg			
Control			20 —	22 000	30 000	3 300	50	50	4,8		
Pyracetam	I	NH_2	25/0,3	26 000	30 000	4 000	58	97	4,0	0	8,0
P-23	I	$N(C_2H_5)_2$	28/0,3	—	—	—	48	90	4,0	0	1,5
P-22	I		30/0,3	—	—	—	52	92	5,2	0	5,0
P-28	I	$NHNH_2$	28/0,1	—	31 500	16 200	75	100	3,8	D	6,0
P-21	I	NHC_6H_5	25/0,1	11 500	28 000	—	25*	40*	18,8*		3,5
P-26	I	OC_2H_5	25/0,5	—	—	—	52	90	5,0	0	6,0
P-24	2	NH_2	30/0,15	20 000	29 000	—	45	75	12,5*	0	6,0
P-27	2	$NHNH_2$	27/0,1	27 000	31 000	—	72	100	3,9	D	6,0
P-25	2	OC_2H_5	22/0,5	—	—	—	50	88	4·9	0	6,0

Legend. 1) Mean values for group of ten mice. 2) Numerator — length of survival in airtight chamber; denominator — threshold dose (in g/kg; increase in length of survival by 20-30%). All parameters in this column differ significantly from control, except P-25. 3) Data for tests with pentobarbital, bicuculline, metrazol, and thiopental sodium were obtained with the compounds in a dose of 300 mg/kg (except EEG). 4) 0 — no marked changes in EEG, S — synchronization, D — desynchronization (doses 20-100 mg/kg). Asterisk — differences significant at the $P < 0.05$ level.

models of single learning of conditioned-reflex passive avoidance (CRPA), using a box with two compartments, one lit, the other dark; when in the latter compartment the animal received painful electrical stimulation [7]; such experiments were carried out on 135 rats. The difference in the length of the animal's stay in the dark compartment before and 24 h after learning was analyzed. Immediately after the end of learning the animal was given an electric shock (ES) inducing retrograde amnesia, and the test compounds or isotonic saline were injected 15-20 sec later.

EXPERIMENTAL RESULTS AND DISCUSSION

As the data in Table 1 show, the test compounds, like pyracetam itself, increased the length of survival of the animals under hypoxic conditions. Judging from the initial doses giving a threshold antihypoxic effect, not all the compounds were equally effective. Substitution of hydrogen atoms attached to the amide nitrogen atom by alkyl radicals (P-22, P-23) led to some reduction of the threshold dose. Substitution of an ester group for the amide group in pyracetam (P-26) and its homolog (P-25) weakened the intensity of the antihypoxic effect. The most definite increase in the antihypoxic effect was observed after lengthening of the side chain (P-24) or substitution of a hydrazide group for the amide group in the molecule of pyracetam (P-28) or its homolog (P-27). These compounds, in a dose of 300 mg/kg, increased the height of the tolerated altitude ceiling compared with the control on average by 550 ± 65 m, whereas pyracetam, in the same dose, did not significantly raise this parameter and in a dose of 500 mg/kg raised it by only 150 ± 52 m. Like pyracetam itself, most of its derivatives that were studied were inactive in neurotropic screening tests. The exceptions were two derivatives containing a piperidine (P-22) and, in particular, a phenyl (P-21) radical in the side chain, and also the hydrazides of pyracetam and its homolog (P-28, P-27). The fundamental difference between the first two compounds and pyracetam is that the latter gives a definite depriming effect (Table 1). A distinguishing feature of the hydrazides

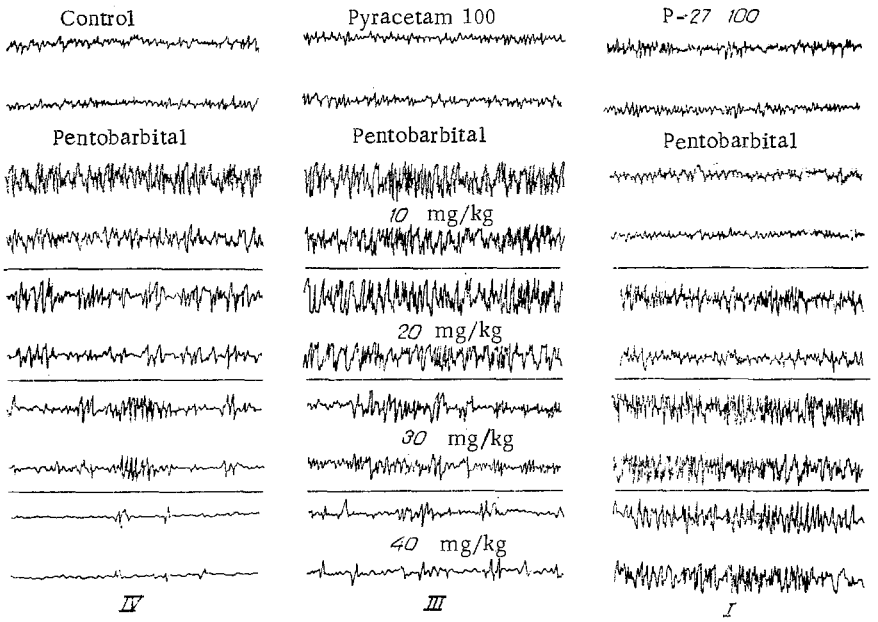


Fig. 1. Effect of pyracetam and its hydrazide derivative on intensity of pentobarbital-induced depression of ECoG. ECoG of left sensomotor and right visual areas of waking rabbit during fractional injection of pentobarbital (10 mg/kg, i.v., at intervals of 2 min) after preliminary (1 h beforehand) injection of physiological saline, of pyracetam (100 mg/kg), or of compound P-27 (100 mg/kg). I, III, IV) Depth of depression of ECoG induced by pentobarbital in animals of corresponding group. Bottom right — calibration: horizontal — 1 sec, vertical — 300 μV. [Calibration originally omitted from the figure — Publisher.]

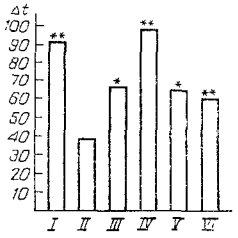


Fig. 2. Comparative activity of pyracetam and its derivatives in rats in the CRPA test. Δt) Difference in length of animal's stay in dark compartment of box before and 24 h after learning during testing of preservation of memory trace (in sec): I) after learning (60 rats); II-IV) after learning, application of ES, and intraperitonal injection of test compounds (15 rats in each group); II) physiological saline, III) pyracetam (200 mg/kg), IV) P-28 (100 mg/kg), V) P-24 (150 mg/kg), VI) P-27 (200 mg/kg). Statistical significance of differences between control and experimental groups estimated by Wilcoxon—Whitney method [1]. *P < 0.05; **P < 0.01 (relative to control series II).

during testing was the presence of a stimulating action, manifested as facilitation of impulse summation in the CNS, together with some potentiation of spontaneous motor activity and, in particular, of motor activity inhibited by pentobarbital (but not by ethanol or chlorpromazine). The antipentobarbital effect of the hydrazides, much stronger than that of pyracetam, was clearly demonstrated in experiments with titration of threshold doses in which pentobarbital induced depression of the ECoG (Fig. 1). Even when pentobarbital was given in a dose of 80 mg/kg together with compound P-27 depression of the ECoG was not deepened more than

before the phase of slow high-amplitude potentials. The pyracetam homolog (P-24), like pyracetam itself [5], strengthened depression of the testing potential in RCEP when the interval between stimuli was 80-125 msec, which characterizes specific potentiation of GABA-ergic inhibition. The phenyl derivative P-21 did not evoke selective potentiation of GABA-ergic inhibition. This fact, and also the character of EEG synchronization induced by P-21, with predominance of spindle-shaped groups and blockade of the arousal reaction to afferent stimulation, suggests that the effect of this compound is mainly subcortical in direction. Hydrazides P-27 and P-28, studied in doses of 50-100 mg/kg, caused activation of the ECoG alternating, if the dose was increased to 400-500 mg/kg, with separate spike-shaped potentials; under these circumstances facilitation of the test potential in RCEP was enhanced.

The results of investigation of activity of the compounds on the performance of the rats in the CRPA test are given in Fig. 2. They show that a single electrical stimulation through the floor was sufficient to produce CRPA: 24 h after learning the length of the animals' stay in the lit part of the box was increased. Retrograde amnesia was manifested by the fact that the animals preferred the dark part of the box. Pyracetam, in a dose of not less than 200 mg/kg, P-24 in a dose of 150 mg/kg, and P-27 in a dose of 200 mg/kg reduced the amnesic effect of ES. The most effective compound was P-28, which completely abolished the action of ES in a dose of only 100 mg/kg.

It follows from these results that some of the pyracetam derivatives studied have the ability to improve consolidation of memory traces when disturbed by ES and of increasing resistance to hypoxia. As was shown previously [6, 8], it is this combination of properties which determines the neurotropic activity of pyracetam, but in the derivatives of pyracetam that were tested this effect varies depending on the length of the side chain and the character of the substituent attached to the nitrogen atom. Introduction of some substituents was accompanied by the appearance not only of neurotropic activity, but also of other types of activity not characteristic of pyracetam. The presence of a depriming action in compound P-21 is evidently due to the lipophilicity of the phenyl radical, endowing it with features of similarity with other phenyl derivatives of GABA-ergic drugs (fenibut, fepyron). When the unique stimulating effect of the hydrazide analogs of pyracetam is interpreted the excitatory properties of some hydrazine derivatives, such as isoniazid, semicarbazides, and so on, must be borne in mind.

The results are evidence that specifically oriented changes in the effects of pyracetam can be brought about and that there are good prospects for the search of pharmacologically active compounds in this series.

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