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NEUROTROPIC ACTIVITY OF PHENYLPYRROLIDONE-2 ISOMERS

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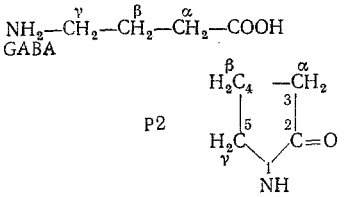
Pyrrolidone-2 (P2) is a cyclic form of gamma-aminobutyric acid (GABA). In experiments on mice and rats the pharmacological activity of three isomers of phenyl derivatives of P2 was investigated. All the compounds inhibited motor activity, lowered muscle tone and body temperature, potentiated the action of hexobarbital, and possessed anticonvulsive (electrical shock, strychnine, metrazol, thiosemicarbazide, audiogenic convulsions) and narcotic activity. The most active isomer was 4-phenylpyrrolidone-2 (phepyron). Comparison of the pharmacological activity of phenyl derivatives of P2 and analogous derivatives of GABA showed that the GABA derivatives have no anticonvulsive or narcotic activity and are less toxic. It is suggested that the phenyl derivatives of P2 are not converted into the analogous GABA derivatives in vivo.

KEY WORDS: gamma-aminobutyric acid; phenylpyrrolidone-2; neurotropic activity.

Pyrrolidone-2 (P2) can be regarded as the cyclic form of gamma-aminobutyric acid (GABA):



The suggestion that P2, which is a nonpolar molecule, is more soluble than GABA in lipids, and so penetrates in greater quantity through the blood–brain barrier, after which it is converted by hydrolysis into GABA, has led many workers to study its pharmacological action. Comparison of P2 with GABA has revealed that it has somewhat greater or less pharmacological activity than P2 depending on the index used [11-13, 15, 16].

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TABLE 1. Comparative Pharmacological Activity of Phenylpyrrolidones (M ± m)

Index	H_2O	3-Phenyl-P2	4-Phenyl-P2	5-Phenyl-P2
Locomotion (no. of crossings of the lines)	34,6±2,9	32,6±5,0	13,2±5,3*	16,2±2,9*
Standing up (number of times)	30,4±2,5	7,8±2,8*	0*	1 3*
Standing up (% of total suppression)	0	0	100*	70*
Muscle tone (% of animals holding on to rod)	100	70	0*	30*
Body temperature, °C	38,6±0,1	34,4±0,9*	28,7±0,4*	32,6±0,7*
Hexobarbital:				
latent period, min	4 1±0 3	4,4±0,3	1,6±0,1*	2,6±0,2*
lateral position, min	80 3±14 3	92,7±11,2	191,5±23,2*	126,8±11,2*
Thiosemicarbazide:				
convulsions, %	100	100	100	100
mortality, %	100	100	80	100
latent period, min	56,0±2,4	84,9±7,0*	190,5±13,4*	107,4±4,5*
length of survival, min	77,9±9,8	120,9±19,1*	227,4±14,7*	115,9±6,7*
Electrical stimulation:				
convulsions, %	100	60	0*	30*
Strychnine:				
convulsions, %	100	87,5	12,5*	87,5
mortality, %	100	75	12,5*	50,0*
latent period, min	7,4±0,4	12,3±1,0*	—	17,0±1,6*
length of survival, min	7,6±0,4	14,3±1,4*	—	20,7±3,6*
Metrazol:				
convulsions, %	100	93	62,5	86,7
mortality, %	100	66,7	26,7*	73,3
latent period, min	4,9±0,4	14,3±1,6	31,1±4,0*	32,2±3,3*
length of survival, min	16,7±3,0	23,4±3,0	40,0±2,7*	42,4±4,3*
LD_{50}, mg/kg			300	480

Legend: 1) compounds injected intraperitoneally into mice in dose of 157 mg/kg 30 min before evaluation of effect. 2) Groups of 10 mice or more. 3) Values for which $P < 0.05$ compared with control marked by asterisk.

TABLE 2. Effect of Phepyron and Phenibut on Motor Activity and Muscle Tone in Mice

Index	ED_{50}, mg/kg, relative to inhibition	
	phepyron	phenibut
Locomotion	84,0 (65,6—107,5)	73,0 (46,0—114,6)
Standing up	64,0 (58,2—70,4)	51,0 (42,4—62,8)
Muscle tone	90,0 (76,3—106,2)	97,0 (78,8—111,2)

However, on local application of P2 to the cerebral cortex, unlike GABA it did not block the excitatory post-synaptic potential [14].

The writer showed previously that phenyl derivatives of GABA have a sedative, tranquilizing, and muscle-relaxing action [4, 5] and that they penetrate into the brain in larger quantities than GABA.

It was accordingly decided to study the changes in the pharmacological properties of P2 after introduction of a phenyl radical into its structure. Since the position of the phenyl radical in the structure of GABA affected the activity of its isomers (of the α, β, and γ derivatives the β isomer – phenibut – was found to be the most active), the pharmacological properties of the corresponding derivatives of P2 also were investigated: 3(α)-phenyl-P2, 4(β)-phenyl-P2, and 5(γ)-phenyl-P2. The compounds were synthesized in the Department of Organic Chemistry, A. I. Gertsen Leningrad Pedagogic Institute.

EXPERIMENTAL METHOD

Noninbred mice and rats of both sexes were used.

To detect pharmacological activity well tried and tested methods were used. The effect on motor activity (locomotion) was assessed by the number of times the animal crossed lines drawn in the form of a cross on the bloor of a transparent plastic box measuring 20× 10× 20 cm during 3 min of observation, and at the same time the number of times the animal stood up on its hind limbs was counted. The effect on coordination and muscle tone was determined by the ability of the mice to hold on to a rod revolving at a speed of 5 rpm for 5 min. The body temperature was measured rectally by the TÉMP-60 thermometer. The effect on the intraperitoneal action of hexobarbital (60 mg/kg) was assessed by the latent period of action and the time in the lateral position. Anticonvulsive activity was determined by the effect on the maximal electroconvulsive seizure (ophthalmic electrodes, current 50 mA, duration of stimulation 0.2 sec), the subcutaneous action of metrazol

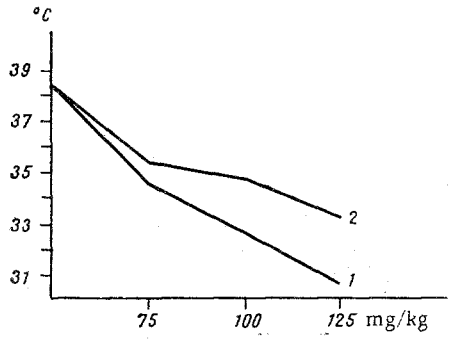

Fig. 1. Effect of phepyron (1) and phenibut (2) on body temperature of mice. Compounds injected 30 min before measurement of temperature. Abscissa, dose of compound (in mg/kg); ordinate, body temperature (in °C).

(90 mg/kg), strychnine (1.5 mg/kg), and thiosemicarbazide (20 mg/kg), and audiogenic convulsions in rats [2]. The test substances were injected intraperitoneally 30-60 min before observation.

The results were subjected to statistical analysis. Values of ED_{50} were determined by the method of Litchfield and Wilcoxon and of LD_{50} by Behrens' method [1].

EXPERIMENTAL RESULTS

The observations showed that P 2, if injected intraperitoneally into mice in a dose of 1000 mg/kg, caused no significant changes in motor activity, muscle tone, or body temperature, had no anticonvulsive action, and did not potentiate the action of hexobarbital. Data showing the pharmacological activity of phenyl derivatives of P 2 are given in Table 1; they show that all the isomers of phenyl-P 2 in a dose of 157 mg/kg had an inhibitory action, which was strongest in the case of 4-phenyl-P 2 (phepyron), which can be regarded as the cyclic form of phenibut.

Comparison of phepyron with phenibut showed both similarities and differences in their action. Both compounds inhibit motor activity, reduce muscle tone, lower the body temperature, and potentiate the action of barbiturates (Table 2).

In a dose of 100 mg/kg both compounds equally potentiate the action of a narcotic dose of hexobarbital (88.9 ± 9.4 and 76.8 ± 7.5 min, compared with 47.0 ± 8.5 min in the control). The hypothermic action of phepyron was rather stronger than that of phenibut (Fig. 1). Neither compound affected the action of amphetamine, oxotremorine, and arecoline. Inhibition of the motor effects (excitation of locomotion, tremor) of these substances was observed only after injection of doses of phepyron and phenibut which increase their ED_{50} with respect to inhibition of motor activity and muscle tone.

Besides the similarities in the action of phepyron and phenibut or, more precisely, of the phenyl derivatives of P2 and GABA, differences also were discovered. All phenyl derivatives of P 2 in a dose of 200 mg/kg or more caused the mice and rats to lie on their sides, with accompanying muscle relaxation. The state of the animals resembled one of narcosis. After intraperitoneal injection of 200 mg/kg of phepyron the mice remained on their sides for 100.0 ± 25.1 and rats for 216.0 ± 21.9 min after latent periods of 2.3 ± 0.2 and 1.3 ± 0.1 min respectively. This short latent period of its action reflects rapid penetration of phepyron into the brain. Neither in this nor in much larger doses did phenibut cause the animals to lie on their sides in a manner characteristic of narcosis. The effects of phenibut, incidentally, developed after a much longer latent period.

Phenyl derivatives of P 2 have anticonvulsive activity (Table 1), whereas phenyl derivatives of GABA, even in large doses, only prolonged the latent period of action of the convulsant factor and the duration of survival of the poisoned animals a little and did not protect them against convulsions or prevent their death [5, 8]. The protective action of phepyron was particularly marked against electrical and strychnine convulsions. ED_{50} for phepyron with respect to suppression of tonic extension of the electroconvulsive fit was 83.0 (55.0-124.0) mg/kg, and of metrazol convulsions 144.0 (124.0-167.0) mg/kg. Phepyron also actively depressed audiogenic con-

vulsions in rats genetically highly sensitive to sound. ED_{50} for phepyron with respect to total suppression of clonico-tonic audiogenic convulsions was 35.0 (24.0-49.3) mg/kg whereas ED_{50} for phenibut was 300.0 (217.0-414.0) mg/kg.

When phepyron was given in doses of up to 100 mg/kg the period of depression of motor activity was preceded by a stage of excitation, characterized by increased but not always coordinated locomotion, without any increase in the number of attempts to stand up. Outwardly the animals' behavior resembled that observed after small doses of sedatives and narcotics. Phenibut as a rule does not cause excitation of locomotion.

Phenyl derivatives of P 2 are much more toxic than phenyl derivatives of GABA. LD_{50} for the former was of the order of 300 mg/kg (Table 1) and for the latter 1000 mg/kg and more [6].

The results of comparison of the pharmacological activity of derivatives of GABA and of its cyclic form (P 2) thus suggest that it is unlikely that P 2 derivatives are converted in vivo into GABA derivatives. This hypothesis is confirmed by the data of Bessman [9], who did not find that GABA was formed from P 2, and of Tower [17], who showed that such conversions take place very slowly in vitro.

When the present investigation was completed the author learned of a paper by Chojnacka-Wojcik et al [10], also devoted to the pharmacology of phenyl derivatives of GABA and P 2. My first results on the pharmacology of the phenyl derivatives of P 2 were announced and published in 1971 [7].

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