PHARMACOLOGICAL ACTIVITY OF OPTICAL ISOMERS OF β-PHENYL-γ-AMINOBUTYRIC ACID

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The pharmacological activity of the racemate of phenigam (β-phenyl-γ-aminobutyric acid) and of its optical isomers with angles of rotation of +3.2 and −3.7° were compared in mice. By all the tests used (effect on motor activity, on movement coordination and muscle relaxation, on the body temperature, and potentiation of the action of hexobarbital) only the D-form was active. The action of the D-form was twice as strong as that of the racemate both by intraperitoneal and by intraventricular injection. The toxicity of the racemate and of the D-isomer of phenigam is the same (1085 and 1025 mg/kg 48 h after intraperitoneal injection). The fact that the activity of the dextrorotatory isomer is twice that of the racemate although its toxicity is not greater suggests that the D-isomer has a stronger therapeutic effect and a greater therapeutic margin of safety than the racemate at present used.

Many biologically active substances containing an asymmetrical atom in their structure have optical isomers of different activity. For instance, the greater activity of L-amino acids than of the D-forms is well known. The substance β-phenyl-γ-aminobutyric acid (phenigam, phenibut), with a sedative and tranquilizing action, which the writer investigated previously [2-4], contains an asymmetrical carbon atom in its structure. It was accordingly postulated that its optical isomers would differ in their pharmacological activity. Optical isomers of phenigam were obtained at the Department of Organic Chemistry of the University of Gdansk, under the direction of Professor G. B. Kupriszewski. These were originally compounds with angles of rotation of +2.2 and −2.0°. Investigation of their pharmacological action showed that the first of these substances possesses slightly greater, and the other slightly less activity than the racemate.

In the investigation described below the pharmacological activity of optical isomers of phenigam with an angle of rotation of +3.2 and −3.7° was studied.

EXPERIMENTAL METHOD

The principal indices of the action of the compounds were the same as those used previously to study phenigam, for they reveal the most characteristic features of its action.

1. Effect on motor activity. The spontaneous motor activity of the muscles was assessed on the basis of locomotion and standing up from a position of recumbency. Locomotion was determined from the number of times the animal crossed lines drawn in the shape of a cross on the floor of a transparent plastic box measuring 20 × 20 × 20 cm during observation for 3 min, and at the same time the number of times the animal got up from recumbency was counted. Both effects were assessed in graduated and alterative (in %, Table 1) forms. Locomotion was also judged from movement of the mice from the center to the periphery of a circular grid 30 cm in diameter. This effect was evaluated in the alternative form (presence or absence).

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TABLE 1. Pharmacological Activity of Optical Isomers of Phenigam (100 mg/kg, intraperitoneally)

Index	Control	± Phenigam	+3,2 Phenigam	−3,7 Phenigam
Locomotion (crossing lines)	30,3±3,1	13,6±3,4	1,1±0,45	20,7±2,4
	100%	100%	50%	100%
Getting up from recumbency . . .	22,3±3,5	—	—	14,2±2,2
	100%	30%	0	100%
Body temperature (in deg)	38,66±0,1	35,14±0,7	31,56±0,51	38,0±0,62
Locomotion (moving across grid) .	100%	60%	0	100%
Rotating rod	100%	50%	0	100%
Body temperature (in deg)	38,76±0,15	33,7±0,5	31,12±0,77	38,18±0,15
Potentiation of action of hexobarbital:				
duration of latent period (in min)	4,4±0,53	2,5±0,17	1,8±0,13	4,3±0,33
duration of lateral position (in min)	36,9±4,9	79,7±4,28	80,7±7,38	35,4±4,7

Note: Underlining with one line means that the value differs significantly from the control ($P < 0.05$); doubled underline means that the difference is significant relative to the control and ± phenigam.

TABLE 2. Comparison of Activity of +3.2 Phenigam and ± Phenigam

Index	Control	± Phenigam (100 mg/kg)	+ 3.2 Phenigam (50 mg/kg)
Locomotion (crossing lines)	28,7±3,3	3,2±1,2	2,9±0,8
Getting up from recumbency	18,3±2,9	0	0
Body temperature (in deg)	38,3±0,2	34,3±0,4	34,1±0,35
Rotating rod	100%	45%	45%
Locomotion (moving across grid)	100%	30%	25%
Potentiation of action of hexobarbital [duration of lateral position (in min)]	43,2±9,2	74,6±5,3	88,4±7,1
ED_{50} calculated from results on rotating rod (in mg/kg)	—	94,0 (78,8—111,4)	44,0 (32,3—59,8)

2. Effect on coordination and muscle tone was determined from the ability of the mice to remain on a rod rotating at 10 rpm for 5 min. This effect was evaluated in the alternative form. In addition, the value of ED_{50} was calculated from this index by the method of Litchfield and Wilcoxon.

3. Effect on body temperature. The temperature was measured rectally by a TÉMP-60 thermometer.

4. Effect on the narcotic effect of hexobarbital. The ability of the compounds to affect the latent period of action of hexobarbital and the duration of the lateral position were investigated. Hexobarbital was injected intraperitoneally in a dose of 60 mg/kg.

5. Toxicity. The effect was calculated 24 and 48 h after intraperitoneal injection into mice. LD_{50} was calculated by Baren's method.

The compounds to be tested were disolved in water and injected intraperitoneally 30-60 min before their effect was determined. In one series of experiments the compounds were injected into the cerebral ventricles by the apparatus designed by Vanecek et al. [5]. The results were subjected to statistical analysis, the graduated results with the aid of Student's t criterion, the alternative by the χ^2 test [1].

TABLE 3. Hypothermic Effect of Phenigam and Its Optical Isomers Given Intraventricular Injection

№	Substance	Dose (in μg)	Number of mice	Original temperature (in deg)	Δt after 45 min	Δt after 90 min
I	Control	—	7	38,97	3,09±0,44	3,34±0,41
II	−3.7 phenigam	10	7	38,63	2,33±0,33	2,49±0,45
III	± phenigam	10	8	38,65	8,05±0,92	9,0±0,99
IV	+3.2 phenigam	5	8	38,65	6,82±0,76	9,22±0,82

Note: $P_{I-II} > 0.1$; $P_{I-III} < 0.001$; $P_{I-IV} < 0.001$; $P_{III-IV} > 0.25$.

EXPERIMENTAL RESULTS

The comparative pharmacological activity of the optical isomers of phenigam and its racemate as shown by these experiments is given in Table 1.

By all indices investigated (except potentiation of the action of hexobarbital) phenigam with an angle of rotation of +3.2° was more effective than the racemate. It inhibited motor activity, disturbed movement coordination and lowered the body temperature to a greater degree. Comparison of the strength of action of the phenigam racemate and its active dextroratory isomer with respect to their effect on motor activity, movement coordination, and body temperature showed that the compound with an angle of rotation of +3.2° is twice as active as the racemate (Table 2).

The levorotatory isomer of phenigam with an angle of rotation of −3.7° was inactive by all the indices.

To compare the penetration of ± phenigam with the penetration of its active and inactive isomers into the brain experiments were carried out with intraventricular injection. The action of the drug was estimated from its hypothermic effect. The results in Table 3 show that +3.2 phenigam, injected intraventricularly, was about twice as active as the racemate, and that the −3.7 isomer had in general no action. Optical isomerism thus did not effect penetration of the compound into the brain.

The toxicity of the racemate and of the active dextrorotatory isomer is identical: LD_{50} for mice by intraperitoneal injection 24 h after injection exceeds 1,400 mg/kg, and 48 h after injection it is 1085 and 1025 mg/kg respectively. It must evidently be assumed that the levorotatory isomer, inactive in all these tests, possesses the same toxicity as the dextrorotatory isomer, because LD_{50} for the racemate and the D-form are identical. This hypothesis could not be verified experimentally because of unavailability of the compound.

The twofold increase in activity of the dextrorotatory isomer without any corresponding increase in toxicity suggests that the D-isomer of phenigam has a stronger therapeutic effect and a wider margin of therapeutic action than the racemate at present in use.

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