SYNTHESIS AND ANTIMONOAMINE OXIDASE ACTIVITY OF AN INDOLE ANALOG OF DEPRENYL

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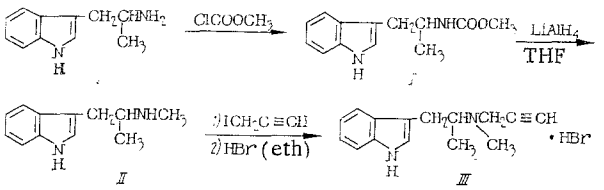
With the aim of studying the mechanism of selective inhibition of mitochondrial monoamine oxidase [amine oxidase (flavin-containing), EC 1.4.3.4] types A and B by propargylamine derivatives, a new compound of this class, 1-indolyl-3-isopropylmethylpropargylamine hydrobromide (the indole analog of deprenyl), was synthesized according to the following scheme:



It has been proposed that for selective inhibition of monoamine oxidase A and B activities, not only the alkylation of the flavin component of monoamine oxidase [7] but also the interaction of the inhibitor with the hydrophobic portion of the enzyme [2, 3], has major significance.

The new compound differs form the well-known propargylamine derivative deprenyl (1-phenylisopropylpropargylamine hydrochloride), a selective inhibitor of monoamine oxidase B [4, 5], only in that the aryl group responsible for hydrophobic interactions with the enzyme [2, 3] is modified, whereas the side chain containing the acetylene group responsible for alkylation of the isoalloxazine ring of the enzyme flavin component is completely preserved. It might be expected that, according to its properties, such an analog of deprenyl will differ substantially from deprenyl itself in its inhibition of monoamine oxidase A and B activities.

In fact, our data indicate that the indole analog of deprenyl displays selective inhibition of monoamine oxidase A, whereas deprenyl [4] mainly inhibits the deamination of 2-phenylethylamine with selective inhibition of monoamine oxidase B.

In pharmacological studies, findings agreeing with the results of biochemical experiments were obtained only with respect to peripheral but not central effects of biogenic monoamines.

EXPERIMENTAL CHEMISTRY

IR spectra were recorded in mineral oil in a UR-10 (GDR) spectrometer; UV spectra were determined in a Specord spectrometer (GDR). Substance individuality and reaction progress were monitored chromatographically on Silufol UV-254 plates (Czechoslovak Socialist Republic) in an isopropanol—aqueous ammonia solution (8:1) system.

1-Indolyl-3-isopropylcarbomethoxyamine (I). To a solution containing 3.43 g (0.02 mole) of α-methyltryptamine [6] in 40 ml chloroform is added an equimolar amount of triethylamine (1.5 ml), and 2.3 ml (0.03 mole) of methyl chlorocarbonate dissolved in 20 ml of chloroform is added dropwise with stirring. After 20 h the solution is washed three times with water,

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then with a 10% HCl solution, then several times more with water until the reaction is neutralized, and the solvent is evaporated. Yield: 4.2 g (90%) of an oily product which crystallizes while standing, mp 93-95°C. Found, %: C, 67.48; H, 6.98; N, 12.11; $C_{13}H_{16}N_2O$. Calculated, %: C, 67.24; H, 6.89; N, 12.50. IR spectrum, ν cm^{-1}: 3350 (N—H), 3290 (N—H), 1670 (CO). UV spectrum, λ_{max} (C_2H_5OH) 280 nm, log ε 1.2.

1-Indolyl-3-isopropylmethylamine (II). To 3 g of $LiAlH_4$ in 50 ml of absolute THF is added dropwise a solution containing 4.1 g of compound I in 50 ml of absolute THF. The mixture is boiled under a dry nitrogen atmosphere for 9-12 h, cooled in an ice bath, and 3 ml of water, 3 ml of a 15% NaOH solution, and 9 ml of water are added sequentially, drop by drop, with stirring. The resulting precipitate is removed by filtration and washed with THF and methylene chloride. The solvent is evaporated. Yield: 3.5 g (95%) of an oily product which crystallizes while standing, mp 91-92°C. The mp according to literature data [7] is 92-93°C. Found, %: C, 75.82; H, 8.01; N, 14.38; $C_{12}H_{16}N_2$. Calculated, %: C, 76.59; H, 8.51; N, 14.89. IR spectrum, ν, cm^{-1}: 3420 (N—H), 3300 (N—H).

1-Indolyl-3-isopropylmethylpropargylamine (III) [8]. To 3.4 g (0.018 mole) of compound II dissolved in 50 ml of anhydrous benzene are added 2.1 ml (0.02 mole) tert-butylamine, and a solution containing 1.82 ml (0.02 mole) propargyl iodide in 20 ml of anhydrous benzene is added dropwise with stirring. The mixture is stirred for 10-15 h. Yield: 3.95 g (95%) of a brown oil.

Compound III (3.95 g) is dissolved in a minimal amount of absolute ether and a saturated hydrogen bromide solution is added dropwise with stirring until a turbidity appears and does not disappear. The mixture is allowed to stand for 5 min, then the sediment is rapidly filtered off and washed several times with ether. mp 184°C (from ethanol). Found, %: C, 55.60; H, 6.42; N, 8.60; Br, 24.31; $C_{15}H_{20}N_2Br \cdot H_2O$. Calculated, %: C, 35.50; H, 6.46; N, 8.60; Br, 24.50. IR spectrum, ν, cm^{-1}: 3250 (N—H), 2680 (N^+—H), 2140 (C≡C).

EXPERIMENTAL BIOCHEMISTRY

White, hybrid male rats weighing 150-180 g were used in the experiments. Liver mitochondrial membrane fragments were isolated according to a previously described method [9] and were stored at −20°C. Monoamine oxidase activity was determined according to the ammonia released when mitochondrial membrane fragments in a sample volume of 1.8 ml were incubated for 20 min at 37°C in an oxygen atmosphere [2] with a substrate added at the following saturating concentrations: 5.7 μmoles of tyramine hydrochloride from Merck (FRG); 10 μmoles of 5-hydroxytryptamine creatinine sulfate from Reanal (Hungary); 0.8 μmoles of 2-phenylethylamine hydrochloride (USSR).

The protein concentration was determined by the colorimetric method of Lowry [10] using crystalline bovine serum albumin as a standard.

After a 20 min preincubation of 0.01 mM 1-indolyl-3-isopropylmethylpropargylamine hydrobromide with mitochondrial membrane fragments at room temperature, the deamination of 5-hydroxytryptamine was almost fully inhibited, whereas the deaminations of tyramine and 2-phenylethylamine were inhibited only by 81 and 56%, respectively, even at an inhibitor concentration of 0.1 mM (see Fig. 1). The use of kinetic method [11] allows a quantitative characterization of the selective inhibition of monoamine oxidase A and B activities by the indole analog of deprenyl.

The kinetic method [11] is used in those cases in which the process of enzyme inhibition proceeds in two stages: a primary (the inhibitor effect is reversible and competitive) and secondary (the inhibitor effect becomes irreversible) step according to the equation:

$$E + I \underset{k_2}{\overset{k_1}{\rightleftarrows}} EI \xrightarrow{k_3} E'I;\ K_i = \frac{k_2}{k_1}, \qquad (1)$$

where K_i is the dissociation constant (in moles) of the intermediate enzyme—inhibitor complex EI, formed in the primary stage of inhibitor action on the enzyme E. The parameter k_3 is the rate constant (in min^{-1}) of EI conversion into an irreversibly blocked enzyme E'I in the secondary stage [11].

Kinetic constants (K_i and k_3) obtained during study of the inhibition mechanism with deprenyl, chlorgyline [12], and the indole analog of deprenyl are presented in Table 1. The data we obtained indicate that the indole analog, as for other propargylamine derivatives

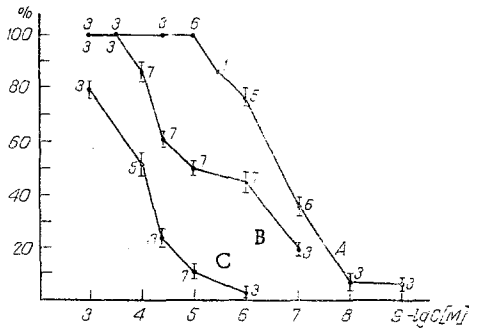

Fig. 1. Inhibition of enzymatic deamination of 5-hydroxytryptamine (A), tyramine (B), and 2-phenylethylamine (C) by 1-indolyl-3-isopropylmethylpropargylamine hydrobromide. Mitochondrial membrane fragments (2.0-2.5 mg protein) were preincubated with the inhibitor concentrations indicated on the abscissa for 20 min at 20°C in 0.1 M phosphate buffer (pH 7.4), after which the reaction was started by the addition of substrate. Along the ordinate are the mean arithmatic values (± average errors) of inhibition (in %) of amine deamination. The number of experiments are indicated in brackets.

TABLE 1. Dissociation Constants K_i (μmoles) of the Intermediate Enzyme–Inhibitor Complexes and Rate Constants k_3 (min^{-1}) for the Irreversible Modification of the Complexes during Inhibition of Monoamine Oxidase A and B Activities by Chlorgyline, Deprenyl, and the Indole Analog of Deprenyl

Monoamine oxidase type	Inhibitor					
	chlorgyline		deprenyl		indole analog of deprenyl	
	K_i	k_3	K_i	k_3	K_i	k_3
A	0,00054	0.05	13.0	0.05	0,094	0,05
B	26,0	0.05	0.11	0.05	24.0	0,06
A:B	0,00002	1	118	1	0.004	0,83

previously studied (chlorgyline, deprenyl [11, 13]), inhibits monoamine oxidase in two stages according to Eq. (1), i.e., a dissociating enzyme–inhibitor complex is formed.

The dissociation constant (K_i) of the intermediate enzyme–inhibitor complex for the indole analog in experiments with 2-phenylethylamine as substrate is 24 μmole, which is 250 times larger than the value (0.09 μmole) in experiments with 5-hydroxytryptamine. Hence, it can be concluded that the indole analog of deprenyl has an affinity for monoamine oxidase A which is approximately 250 times greater than that for monoamine oxidase B. However, it is surpassed by chlorgyline, which has an even greater affinity (approximately 50,000-fold) for monoamine oxidase A.

Comparison of K_i values found previously [12] for deprenyl (0.11 and 13 μmole in experiments with 2-phenylethylamine and 5-hydroxytryptamine, respectively) indicates that deprenyl has an approximately 118-fold higher affinity for monoamine oxidase B.

The stage of irreversible inactivation of monoamine oxidases A nad B by the indole analog proceeds at about the same rate, judging by comparison of k_3 values. The k_3 values are 0.05-0.06 min^{-1}, which correspond to those obtained previously for deprenyl and chlorgyline.

Thus, substitution of the phenyl residue in deprenyl by an indole residue leads to a change in the selectivity of inhibition of monoamine oxidase A nad B activities. This suggests the importance of hydrophobic interactions during inhibition of monoamine oxidase by propargylamine derivatives [2, 3] and supports a hypothesis in which the selectivity of monoamine oxidase A and B inhibition is determined at the stage of dissociable enzyme–inhibitor complex formation [12].

EXPERIMENTAL PHARMACOLOGY

Pharmacological study of the indole analog of deprenyl was conducted according to the following parameters: 1) LD_{50} in white mice after injection; 2) influence on the depressive effects of tetrabenazine in mice; 3) influence on head shaking in mice caused by 5-hydroxytryptophan; 4) influence in the temperature effect of L-DOPA and 2-phenylethylamine in mice; 5) effect on the arterial pressure and nictitating membrane responses when serotonin, tyramine, and 2-phenylethylamine are administered to narcotized cats.

The LD_{50} of the indole analog (III) is 550 mg/kg in white mice. At doses equal to 1/10-1/5 of the LD_{50} (50-100 mg/kg), III caused increased motor activity, at higher doses (250-400 mg/kg), tremors, the appearance of stereotype, and marked motor excitation, and at toxic doses (500 mg/kg and above), clonic spasms.

Compound III decreased the depressive effort of tetrabenazine (hypothermia and blepharoptosis) in white mice. At doses of 5, 10, and 25 mg/kg, the blepharoptosis caused by tetrabenazine decreased from 2.4 ± 0.36 points to 2.0 ± 0.2 ($P > 0.05$), 0.6 ± 0.18 ($P < 0.001$), and 0.3 ± 0.18 points ($P < 0.001$), respectively (measurements were taken 1 h after ip injection of 40 mg/kg tetrabenazine; compound III was given 1.5 h before tetrabenazine).

Compound III increased the spasmodic activity of 5-hydroxytryptophan in mice. With preliminary (1.5 h before) administration of compound III, 50 mg/kg ip 5-hydroxytryptophan, which usually does not change the state of the animals, caused head shaking.

When compound III was given in doses of 5.0, 10, and 25 mg/kg, 5-hydroxytryptophan caused head shaking in 45, 60, and 90% of the mice, respectively. Compound III also strengthened the effect of L-DOPA. When compound III was given 1.5 h in advance, L-DOPA (200 mg/kg given ip), which causes a hypothermal effect in mice, caused hyperthermia which is characteristic for large doses of L-DOPA (400-500 mg/kg). In control mice the rectal temperature 30 min after injection of 200 mg/kg L-DOPA was 33.9 ± 0.42°C, and under the effect of compound III at doses of 5, 10, and 25 mg/kg temperatures were 36.4 ± 0.6°C ($P < 0.02$), 38.4 ± 0.53°C ($P < 0.001$), and 40.2 ± 0.24°C ($P < 0.001$), respectively.

The indole analog was also able to increase the effects of 2-phenylethylamine, in particular, the hyperthermal effect. With preliminary (1.5 h) injection of III in doses of 1, 5, and 10 mg/kg, 2-phenylethylamine (25 mg/kg given ip), which has little effect on the body temperature of mice, caused hyperthermia up to 36.2 ± 0.46°C ($P > 0.05$), 37.2 ± 0.2°C ($P < 0.01$), and 37.1 ± 0.39°C ($P < 0.05$), respectively. The rectal temperature of control mice was 35.5 ± 0.14°C. Deprenyl (10 mg/kg) increased the temperature to 39.0 ± 0.39°C ($P < 0.001$).

The influence of III on the peripheral effects of biogenic amines (arterial pressure response and constriction of nictitating membrane) was studied in experiments on cats. An increase in the effects of serotonin was observed 1 and 2 h after iv injection of 5 mg/kg of III, whereas the effects of tyramine and 2-phenylethylamine were practically unchanged. These results agree with those of biological studies carried out in rat liver mitochondria.

However, in pharmacological experiments concerning the influences on biogenic amines of the brain (increase of central effects of the biogenic amine precursors, 5-hydroxytryptophan and L-DOPA, and increase of the effect of 2-phenylethylamine), the indole analog of deprenyl did not display a very high or selective inhibition of oxidative deamination of these or other amines. The compound potentiated the effects of 5-hydroxytryptophan at significantly higher doses than those for the selective monoamine oxidase A inhibitor, harmine (ED_{50} of harmine in analogous experiments is 55 mg/kg when administered subcutaneously [14]), or chlorgyline [15].

Moreover, compound III increased the effect of 2-phenylethylamine, a substrate of monoamine oxidase B, but to a much smaller extent than the monoamine oxidase B inhibitor, deprenyl.

Thus, compound III, an indole analog of deprenyl, is inferior to known monoamine oxidase inhibitors according to its monoamine oxidase-inhibiting activity in pharmacological experiments.

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CLINICAL PHARMACOKINETICS AND BIOPHARMACEUTICAL INVESTIGATION OF PYRACETAM

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Pyracetam (nootropyl, N-acetamido-2-pyrrolidone) is the first psychotropic drug assigned to the new pharmacological class of nootropic agents [1, 2]. Attempts were made previously [3, 4] to describe the dynamics of the pyracetam concentration in the blood of patients with a one-part pharmacokinetic model. Using this model only a portion of the exponential decrease in the concentration of the preparation in the so-called β-phase could be satisfactorily described. However, the processes of absorption and distribution of the drug among organs and tissues cannot be quantitatively characterized by this method. In this work the entire pharmacokinetic curve and the main processes occurring with the drug in the human organism were characterized quantitatively using a two-part mathematical model. A sensitive micromethod of analysis of pyracetam in blood serum was developed for a simplification and facilitation of the investigation. For this method 0.5-0.8 ml of blood is sufficient (instead of 6.0 ml of blood according to the well-known method). The more adequate model and simplified method of analysis have made it possible to study the kinetics of the absorption of pyracetam from capsules with various fillers (magnesium carbonate, lactose) and also to investigate the influence of the taking of the food on the process of absorption of the drug.

MATERIALS AND METHODS

Various drug forms of pyracetam were used: 1) gelatin capsules with magnesium carbonate as a filler (developed at the S. Ordzhonikidze All-Union Scientific-Research Institute of the Chemical and Pharmaceutical Industry); 2) gelatin capsules with lactose as a filler (produced by UCB, Belgium); 3) a 10% aqueous solution internally; 4) ampuls for intravenous injection.

We investigated 11 patients (8 men and 3 women), each of whom received 800 mg pyracetam once on an empty stomach 1.5 h before breakfast, in the form of: a) capsules (2 × 400 mg) with magnesium carbonate; b) capsules (2 × 400 mg) with lactose; c) aqueous solution internally; d) intravenous injection. In another series of investigations the same patients received 800 mg of the drug in the form of capsules (2 × 400 mg) with magnesium carbonate 1 h after a standard breakfast. There was a 3-4 day interval between the investigations of different drug forms. After coagulation of blood specimens and centrifugation (10 min, 6000 rpm), the serum was separated; 5 ml of ethanol was added to 0.2-0.4 ml of serum (precise volume) to precipitate proteins, the mixture shaken for 5 min and centrifuged for 15 min at

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