INHIBITION OF MITOCHONDRIAL MONOAMINE OXIDASE BY 3,4-DIMETHOXYBENZYLHYDRAZINE (VETRAZIN) AND ITS RELATED COMPOUNDS

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The compound 3,4-dimethoxybenzylhydrazine (vetrazin), whose synthesis and pharmacological properties were described previously [6, 7], possesses the power of inhibiting monoamine oxidase activity [8]. Vetrazin has relatively low toxicity and a broad spectrum of therapeutic activity [7].

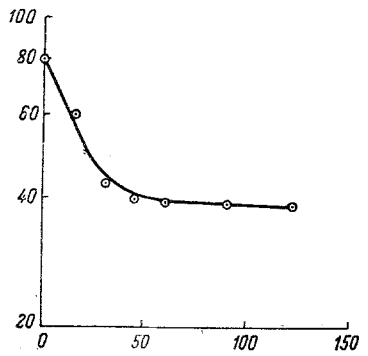

Fig. 1. Effect of duration of preincubation on inhibition of monoamine oxidase of mitochondria of rats' liver by vetrazin. Samples containing 10 mg each of a lyophilized preparation of monoamine oxidase [4] from the mitochondria of a rat's liver (suspension in 0.1 M potassium-phosphate buffer solution at pH 7.4) were preincubated at room temperature with vetrazin (final concentration 1×10^{-5} M). Substrate—tyramine (final concentration 1×10^{-3} M). Along the axis of abscissas—duration of preincubation (in min), along the axis of ordinates—residual activity (in %, logarithmic scale).

In this paper we describe data relating to the kinetics of inhibition of the mitochondrial monoamine oxidase activity of the liver and brain of rats in vitro by vetrazin and certain related compounds.

METHOD

The preparations of mitochondrial monoamine oxidase of the brain and liver of rats were partially purified by the method of Seiden and Westley [13] or by a technique devised by ourselves [2]. According to our findings, these preparations were indistinguishable as regards sensitivity to the inhibitors tested from monoamine oxidase solubilized by the action of ultrasonic waves. The conditions of determination of the monoamine oxidase activity by the ammonia split off amines in the course of incubation by the enzyme preparations have been described previously [4]. The substrates were hydrochlorides of tyramine (T. Schuhardt), benzylamine (Khar'kov Factory for Chemical Reagents, 3-hydroxytyramine (dopamine) (Calbiochem), serotonin creatininesulfate (T. Schuhardt), and DL-noradrenalin bitartrate (Light).

RESULTS

It is clear from Fig. 1 (1 of 3 analogous experiments) that maximal inhibition of monoamine oxidase activity by vetrazin was obtained during the first 30 min of preincubation. A similar phenomenon was found during the study of the kinetics of inhibition of monoamine oxidase activity by other aromatic alkylhydrazines [12]. Preincubation of the enzyme preparations with vetrazin in an

TABLE 1. Inhibition by Vetrazin of Oxidative Deamination of Amines under the Influence of Mitochondrial Monoamine Oxidase of Rat Liver and Ox Brain*

Amines	Liver		Brain	
	K_m	I_{50}	K_m	I_{50}
Tyramine	$1.25 \cdot 10^{-3}$	$2.5 \cdot 10^{-5}$	$8.2 \cdot 10^{-2}$	$2.35 \cdot 10^{-5}$
Benzylamine	$1.8 \cdot 10^{-2}$	$3.15 \cdot 10^{-5}$	–	–
Serotonin	$1 \cdot 10^{-2}$	$3.55 \cdot 10^{-5}$	$3.8 \cdot 10^{-2}$	$1.4 \cdot 10^{-5}$
Noradrenalin	$1.2 \cdot 10^{-2}$	$2 \cdot 10^{-6}$	–	–
Dopamine	–	–	$5.2 \cdot 10^{-2}$	$3.29 \cdot 10^{-5}$

*Samples each containing 5 mg (liver) or 50 mg (brain) of lyophilized preparations of monoamine oxidase were preincubated for 30 min at room temperature with vetrazin in concentrations necessary for inhibition of enzyme activity by 10-90%. After addition of saturating concentrations of substrates, the samples were incubated and the amount of liberated ammonia was determined as described earlier [5].

TABLE 2. Effect of Vetrazin and Certain Related Compounds on Monoamine Oxidase Activity of Mitochondria Rats' Liver*

Compound No.	Conventional name	Rational name	I_{50}
1	Vetrazin	3,4-Dimethoxybenzylhydrazine	$2.5 \cdot 10^{-5}$
2	TM-1	2,5-Dimethoxybenzylhydrazine	$9 \cdot 10^{-6}$
3	TM-2	3,4-Dimethoxyphenylethylhydrazine	$1.5 \cdot 10^{-5}$
4	TM-3	2,3,4-Trimethoxy-β-phenylethylhydrazine	$9 \cdot 10^{-6}$
5	TM-4	4-Methoxybenzylhydrazine	$2 \cdot 10^{-6}$

*Samples each containing 10 mg of a lyophilized preparation of monoamine oxidase 41 from the mitochondria of rats' liver (suspension in 0.1 M potassium-phosphate buffer solution at pH 7.4) were preincubated for 30 min at room temperature with vetrazin (final concentration $1 \cdot 10^{-5}$ M). Substrate—tyramine (final concentration $5 \cdot 10^{-3}$ M). All compounds were hydrochloride salts.

atmosphere of oxygen or hydrogen had the same effect on the deamination of tyramine as preincubation in an atmosphere of air. Replacement of the air by nitrogen did not depress the inhibitory action of vetrazin on monoamine oxidase activity. In this respect vetrazin differed from 1-isonicotinoyl-2-iospropylhydrazine (iprazid, iproniazid) [10] but was similar to trans-2-phenylcyclopropylamine (transamine, parnat) [15].

The concentration of vetrazin required to inhibit the oxidative deamination of noradrenalin by mitochondrial monoamine oxidase of rats' liver was one order lower than the values of I_{50} for the other amines investigated (Table 1). In the experiments with mitochondrial monoamine oxidase from ox brain this selective inhibition of deamination of dopamine did not take place.

Our results showed that the addition of excess of substrate to the sample immediately after the addition of vetrazin abolished the inhibitory action of the latter. In the course of preincubation, however, vetrazin acquired the properties of a typical noncompetitive inhibitor of monoamine oxidase (Fig. 2). A similar pattern was found during the kinetic investigations of the inhibition of monoamine oxidase activity by hydrazines [3, 10] and aromatic alkylhydrazines [12], and also in experiments to study the inhibition of oxidation of tyramine by trans-2-phenylcyclopropylamine [15]. The latter compound, however, exhibited the properties of a noncompetitive monoamine oxidase inhibitor if 4-phenyl-n-butylamine was used as substrate [15]. According to our findings, after preincubation

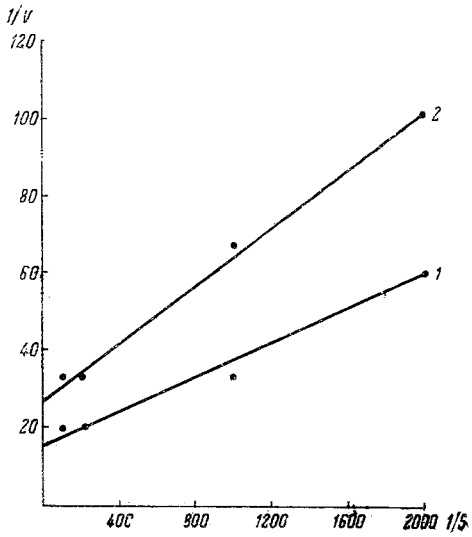

Fig. 2. Effect of vetrazin on oxidative deamination of tyramine by mitochondrial monoamine oxidase from liver of rats. The results have been plotted on the graph by the method of Lineweaver and Burk [15]. 1) Without inhibitor; 2) in the presence of vetrazin in a concentration of $2.5 \cdot 10^{-5}$ M (duration of preincubation with enzyme 30 min at room temperature).

for 30 min with mitochondrial monoamine oxidase of rats' liver, vetrazin noncompetitively inhibited the oxidative deamination, not only of tyramine (Fig. 2), but also of serotonin, benzylamine, and dopamine (in the last case the source of the enzyme was mitochondrial monoamine oxidase of ox brain).

Experiments to study the reversibility of the action of vetrazin on monoamine oxidase in vitro, carried out by the method of Udenfriend and co-workers [14], showed that after preincubation for 30 min with preparations of mitochondrial monoamine oxidase of rat's liver, vetrazin caused irreversible inhibition of the oxidative deamination of tyramine.

The mean results of 3 experiments to investigate the action of certain compounds related to vetrazin on the oxidative deamination of tyramine by mitochondrial monoamine oxidase of rats' liver, given in Table 2, show that 4-methoxybenzylhydrazine (compound 5) inhibited its activity by 50% in a concentration one order lower than the value of I_{50} for vetrazin.

On the basis of results indicating the role of cations of metals in the catalytic activity of mitochondrial monoamine oxidases [1], it has been suggested [5] that the primary stage in the action of certain inhibitors on monoamine oxidase consists of their linking with the metal in the catalytic center of the enzyme. According to R. S. Krivchenkova's findings, obtained in our laboratory by the method of determination of the relative indices of instability of complex compounds [5], vetrazin possesses the power of binding cations of metals. If our hypothesis of the mechanism of the primary stage of the action of hydrazine derivatives on monoamine oxidase is correct, a reduction in the inhibitory action of vetrazin on the activity of this enzyme is to be expected in the presence of metallic cations. The results of preliminary experiments with mitochondrial monoamine oxidase from rats' liver support this hypothesis. In the presence of the cations Cu^{++} or Ni^{++} in concentrations having no effect on the activity of monoamine oxidase, the inhibition of the oxidative deamination of tyramine by vetrazin was depressed on the average by 20%. After preincubation of vetrazin with monoamine oxidase, the addition of metals had no effect on the degree of inhibition of the activity of the enzyme. Investigations of the mechanism of action of vetrazin and other monoamine oxidase inhibitors on the activity of the mitochondrial amine oxidases are proceeding.

LITERATURE CITED

1. V. Z. Gorkin, In book: Abstracts of Sectional Proceedings of the 5th International Biochemical Congress [in Russian], 1, Moscow (1962), p. 251.
2. V. Z. Gorkin and I. V. Verevkina, Vopr. med. khimii, No. 3 (1963), p. 315.
3. V. Z. Gorkin, L. I. Gridneva, L. A. Romanova, et al., Biokhimiya, No. 6 (1962), p. 1004.
4. V. Z. Gorkin and L. A. Romanova, Biokhimiya, No. 5 (1959), p. 826.
5. V. Z. Gorkin, R. S. Krivchenkova, and I. S. Severina, Abstracts of Proceedings of a Scientific Conference on the Mechanism and Kinetics of Enzymic Catalysis [in Russian], Moscow (1963), p. 10.
6. A. N. Kost, R. S. Sagitullin, and M. A. Yurovskaya, Zh. obshchei Khimii, No. 6 (1963), p. 2011.
7. A. N. Kudrin, G. S. Koroza, A. N. Kost, et al., Farmakol. i toksikol., No. 1 (1963), p. 75.
8. A. N. Kudrin and A. N. Kost, In book: Adrenalin and Noradrenalin [in Russian], Moscow (1964), p. 131.
9. V. I. Kuznetsov, Zh. neorgan. khimii, No. 5 (1961), p. 1042.
10. A. N. Davison, Biochem. J., 67 (1957), p. 316.
11. M. Dixon and A. Webb, Enzymes [Russian translation], Moscow (1961).
12. A. L. Green, Biochem. J., 84 (1962), p. 217.
13. L. S. Seiden and J. Westley, Biochim. biophys. Acta, 58 (1962), p. 363.
14. S. Udenfriend, B. Witkop, B. G. Redfield, et al., Biochem. Pharmacol., 1 (1958), p. 160.
15. E. A. Zeller and S. Sarkar, J. biol. Chem., 237 (1962), p. 2333.