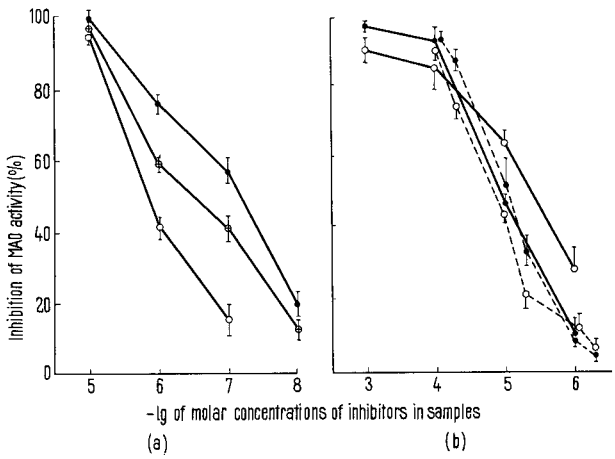
Effect of Some Monoamine Oxidase Inhibitors on Deamination of Biogenic Monoamines by Rat Liver Mitochondrial Monoamine Oxidase

Harmine and 1-isonicotinoyl-2-(benzylcarboxamidoethyl) hydrazin (Nialamid) were recently shown[1-3] to inhibit the deamination of various monoamines in vitro more or less selectively; iproniazid (1-isonicotinoyl-2-isopropylhydrazin), trans-2-phenylcyclopropylamine (Parnate) and β-phenylisopropylhydrazin (Catron) do not possess this ability[3,4]. We now report data on the effects of N-methyl-N-benzylpropynylamine (Pargyline MO 911 I), β-phenylethylhydrazin (Nardil II) and 1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl hydrazin (isocarboxazid Marplan III)[5].

Rat liver mitochondria extracted by hypotonic phosphate buffer[6] and solubilized[4] by a non-ionic detergent OP-10 were used as crude preparations of monoamine oxidase (MAO). Preparations purified about 25-fold as compared with the crude ones were prepared by chromatography on brushite columns[7].

Concentrations of I (Figure a) inhibitory for deamination of dopamine, and especially for tyramine, are significantly lower than those required for inhibition of serotonin deamination (pI_{50} values are 6.42 ± 0.69, 7.07 ± 0.11 and 5.64 ± 0.44, respectively). II (Figure b) inhibits the deamination of serotonin in significantly lower concentrations than the deamination of tyramine (pI_{50} values are 5.46 ± 0.06 and 4.88 ± 0.04, respectively $P < 0.001$). No significant difference in respective values could be found in similar experiments with III (pI_{50} values are 4.95 ± 0.4 and 4.86 ± 0.39, $P > 0.6$).

Irreversible inhibition of deamination of tyramine by I, developing during preincubation of MAO with the inhibitor in aerobic conditions[8], is prevented by 8-hydroxyquinoline (Table) – a chelating agent reversibly and competitively inhibiting MAO due to interaction with metal ions essential for activity of the enzyme[9,10]. Harmine – reversible inhibitor of MAO which possibly interacts with the flavin component of the enzyme[11] – does not cause this effect. Quite different results were obtained in similar experiments with serotonin as a substrate[8].



Inhibition of enzymatic deamination of monoamines by (a) I and (b) II (solid line) or III (dotted line). Experimental conditions – see [1]. I was preincubated for 60 min at 20°C with a purified MAO (0.5 mg of protein per sample), isocarboxazid – for 45 min at 20°C with 'crude' MAO, II was tested without preincubation. Tyramine (●), serotonin (o) or dopamine (⊕) were added in amounts of 5.76, 9.89 or 6.00 μmoles per sample, respectively. Each value represents the mean of 3–4 experiments. Vertical lines: standard error

Complete inhibition of enzymatic activity of MAO by preincubation with I does not decrease the content of SH-groups in purified MAO preparations[8,12]. These preparations are especially sensitive as compared with crude MAO towards the inhibitory effect of mercaptide-forming reagents, but their sensitivity towards the inhibition by pargyline does not differ from that of the crude enzyme[12]. These data do not support the hypothesis[11] that SH-groups may be a primary site of action of I on MAO in vitro.

Effect of 8-hydroxyquinoline and harmine on inhibition by I of tyramine deamination in vitro. Experimental conditions – see [1] and legend to Figure. Final concentrations of I, 8-hydroxyquinoline and harmine in samples were 10^{-7} M, $2.5 \cdot 10^{-4}$ M and 10^{-4} M, respectively. Dialysis against 500-fold volume of 0.01 M phosphate buffer, pH 7.4, was carried out during 24 h. Values represent means of 4 parallel experiments

Sample No.	Treatment of purified MAO preparation	Inhibition of tyramine deamination, %
1.	Preincubation with I	62.5 ± 7.5
2.	Preincubation with I; dialysis	70.4 ± 1.6
3.	Addition of 8-hydroxyquinoline; dialysis	0
4.	Addition of 8-hydroxyquinoline; preincubation with I; dialysis	10.0 ± 2.5
5.	Addition of harmine; dialysis	0
6.	Addition of harmine; preincubation with I; dialysis	81.0 ± 1.0 81.0 ± 1.0

Выводы. N-метил-N-бензилпропиниламин тормозит дезаминирование тирамина митохондриальной МАО в концентрациях, значительно более низких, чем те, которые необходимы для торможения дезаминирования серотонина.

V. Z. Gorkin, L. I. Gridneva, L. B. Klyashtorin, I. V. Veryovkina, and I. Vina

Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR (Moscow), August 30, 1965.

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