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ON THE SELECTIVE INHIBITION BY SOME MONOAMINE OXIDASE INHIBITORS OF DEAMINATION OF BIOGENIC MONOAMINES *IN VIVO*

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Abstract—Pargyline previously shown to be *in vitro* a selective inhibitor of deamination of tyramine by rat liver mitochondrial MAO causes *in vivo* a distinct selective inhibition of tyramine deamination in liver, kidney, and brain tissues of rat. No correlation could be found between the results of similar *in vitro* and *in vivo* experiments with phenelzine and nialamide. Modaline exhibits certain selectivity in its inhibitory effect *in vivo* on deamination of tyramine as compared with that of serotonin in liver and brain (but not in kidney) tissues; the compound Ro 4-2308, to the contrary, causes statistically significant prevailing inhibition *in vivo* of enzymatic deamination of serotonin in liver. Selective inhibition by MAOI of enzymatic deamination of various biogenic monoamines *in vivo* is possible but the patterns of this selectivity in various tissues could not be predicted on the basis of *in vitro* experiments with a standard preparation of liver mitochondrial MAO.

PREVIOUS work[1–5] showed that some monoamine oxidase inhibitors (MAOI) possess the ability to cause more or less selective inhibition of oxidative deamination (catalyzed by rat liver mitochondrial monoamine oxidase) of some biogenic monoamines *in vitro*. Reactions of enzymatic deamination of tyramine and serotonin (5-hydroxytryptamine) as compared with oxidative deamination of other biogenic monoamines exhibit the most distinct difference in sensitivity towards the inhibitory effect of various compounds.[2, 6, 7]

The purpose of present work was to find out whether the potent MAOI used in clinical medicine and/or in pharmacological investigations possess the ability to inhibit selectively deamination of tyramine and serotonin in various tissues under conditions of *in vivo* experiments.

MATERIALS AND METHODS

Pargyline (*N*-methyl-*N*-benzylpropynilamine.HCl) was a gift by Professor T. L. Sourkes (Allan Memorial Institute of Psychiatry, Montreal, Canada) and Mrs. I. Vina (Institute of Organic Synthesis, Riga, U.S.S.R.). Phenelzine (β-phenylethylhydrazine. HCl; nardil) was synthesized by Dr. R. S. Sagitullin (Chemical Faculty, Moscow State University). Nialamide (1-isonicotinoyl-2-(benzylcarboxamidoethyl) hydrazine) was a product prepared by Pfizer Corporation, Brussels, under authority of Chas.

Abreviations used in the text: MAO = monoamine oxidase; MAOI = monoamine oxidase inhibitors.

Pfizer and Co., New York and was obtained commercially. Modaline (2-methyl-3-piperidinopyrazine sulfate) was provided by the Warner-Lambert Research Institute, Morris Plains, New Jersey, research affiliate of Warner-Chilcott Laboratories. The compound Ro 4-2308 (N^1-*d*-hydroxypropionyl-N^1,N^2-di-isopropyl-hydrazine) was kindly presented by Professor A. Pletscher, Director of the Medical Research Department, F. Hoffmann—La Roche and Co., Basle, Switzerland.

Aqueous solutions of the inhibitors were carefully neutralized and injected subcutaneously into male white rats (160–180 g) which were starved for 18 hr before the beginning of the experiment. Control rats were injected by equal volumes of 0·9 per cent sodium chloride solution. The animals were sacrificed within two hours after a single injection. Liver, brain, and kidney were quickly removed, washed in ice-cold 0·9% sodium chloride solution, blotted, and stored in a refrigerator at −25° if necessary.

The tissues were homogenized in an all-glass Potter–Elvehjem homogenizer with an equal (by weight) volume of 2·5% solution of a non-ionic detergent OP-10 (ref. 8) in 0·2 M phosphate buffer, pH 7·45. The homogenates in amounts equivalent to about 300 mg of fresh tissue were introduced in samples for estimation of MAO activity. Content of protein in the samples was determined by Kjeldahl method.

Samples for estimation MAO activity contained in a total volume of 1·8 ml (0·1 M K-phosphate buffer, pH 7·4) either tyramine hydrochloride or serotonin creatinine-sulfate (final concentrations 3×10^{-3} M or 5×10^{-3} M, respectively) and were incubated for 45 min at 37° in oxygen. Both substrates were chemically pure products by Theodor Schuchardt, B.R.D. Fixation of samples by addition of trichloroacetic acid and determination of ammonia (liberated in course of incubation) by means of isothermic distillation in Conway units with subsequent nesslerization were as described previously.[9]

In control animals activity of MAO (expressed in μmoles of ammonia liberated within 45 min incubation per mg of protein) in tissues of liver, brain, and kidney was respectively (with tyramine as a substrate): 0·59 ± 0·03, 0·47 ± 0·04 and 0·19 ± 0·04 or (with serotonin as a substrate): 0·41 ± 0·03, 0·38 ± 0·02 and 0·21 ± 0·04.

RESULTS

For presentation in the Table 1 we have choosen only the results of experiments carried out with those doses of MAOI which are required to cause about 50 per cent inhibition of oxidative deamination of at least one of the monoamines.

Pargyline within 2 hr after a single injection causes highly significant selective inhibition of deamination of tyramine as compared with that of serotonin in all the three tissues studied. Phenelzine is highly effective under similar experimental conditions as an inhibitor of rat kidney MAO causing significantly more distinct blocking of tyramine (then of serotonin) deamination in this tissue. In liver however inhibition by phenelzine of deamination of serotonin prevailed over that of tyramine deamination. In brain tissues phenelzine inhibits the oxidative deamination of both amines to exactly the same degree. We were unable to observe *in vivo* any selectivity in inhibition by nialamide of deamination of tyramine and serotonin. Modaline inhibits mainly the deamination of tyramine in liver and brain (but not in kidney) of rats. Ro 4-2308 exhibits moderate but statistically significant selectivity in inhibition of deamination

TABLE 1. INHIBITION BY SOME MONOAMINE OXIDASE INHIBITORS OF DEAMINATION OF TYRAMINE AND SEROTONIN IN RAT LIVER, BRAIN AND KIDNEY *in vivo*

Monoamine oxidase inhibitors	Doses mg/kg	Liver	Brain	Kidney
Pargyline (*N*-methyl-*N*-benzylpropynylamine·HCl)	2·5	43 ± 4·5 (5)*	46 ± 1·4 (4)*	55 ± 3·8 (3)*
		14 ± 1·8 (5)	10 ± 0·9 (5)	6 ± 2·9 (5)
Phenelzine (β-phenylethylhydrazine·HCl)	0·3			71 ± 2·1 (3)
				52 ± 1·9 (3)
	2·5	33 ± 3·7 (3)‡	75 ± 4·1 (5)	
		55 ± 2·4 (3)	75 ± 6·0 (5)	
Nialamide (1-isonicotinoyl-2-(benzylcarboxamidoethyl) hydrazine)	10·0			72 ± 3·0 (3)
				69 ± 1·7 (4)
	15·0	64 ± 5·9 (5)		
		76 ± 4·5 (5)		
	30·0		0 (4)	
			1 ± 1·05 (3)	
Modaline (2-methyl-3-piperidinopyrazine sulfate)	2·0			53 ± 2·7 (3)
				56 ± 2·2 (3)
	2·5	87 ± 0·7 (3)†	70 ± 4·3 (5)‡	
		73 ± 3·9 (4)	48 ± 2·8 (5)	
Ro 4-2308 (*N'-d*-hydroxypropionyl-N^1,N^2-di-isopropylhydrazine)	3·0			51 ± 3·9 (3)
				46 ± 5·9 (3)
	7·0	45 ± 2·0 (4)‡	74 ± 2·5 (5)*	
		55 ± 1·6 (4)	59 ± 1·8 (5)	

Figures express the mean values ±S.E. (number of experiments, in parentheses) of inhibition of deamination of tyramine (above the line) or of serotonin (under the line) in per cent as compared with respective control samples.

Criteria of statistical significance (by Student's *t*-test) of difference between the values for per cent of inhibition of deamination of tyramine and serotonin:

* $P < 0.001$; † $0.001 < P < 0.002$; ‡ $0.002 < P < 0.01$.

of serotonin as compared with that of tyramine only in liver but neither in kidney nor in brain tissues.

DISCUSSION

Our studies on the problem of "multiplicity" of mitochondrial monoamine oxidases[1, 10] prompted us to investigate the inhibitory effect *in vitro* of a number of compounds on deamination of various monoamines.[2–4] Proflavine (3,6-diaminoacridine sulfate) possesses a marked ability to inhibit selectively oxidative deamination of serotonin as compared with the deamination of some other monoamines.[2] Another acridine derivative—acridine orange NO—exhibits a similar effect but a xanthene derivative-pyronine G (in the tricyclic structure of which oxygen but not nitrogen is a heteroatom), to the contrary, inhibits oxidative deamination of tyramine in significantly lower concentrations than those required for inhibition of serotonin deamination.[3] Studies on the effect of some tricyclic dyes on deamination of various monoamines *in vivo* carried out in our Laboratory[11] established that acridine orange NO and pyronine G possess the ability to inhibit selectively the deamination of tyramine and serotonin in rat liver and brain tissues in accordance with those patterns of selectivity which were observed in our experiments with rat liver mitochondrial MAO *in vitro*. Striking selectivity of the inhibitory effect of a naturally occurring tricyclic compound—harmine (3-methyl-II-methoxy-β-carboline) on the deamination of serotonin by rat

liver mitochondrial MAO[5, 7] is observed also *in vivo* both in rat liver and brain tissues.[12] Similar correlation has been observed,[13] between the results of experiments on selective inhibition by α-methyltryptamine and its derivatives of serotonin deamination *in vitro* and *in vivo*.

The data presented in this paper show that some MAOI used in clinical medicine or (and) in experimental investigations are able to inhibit *in vivo* more or less selectively the deamination of tyramine and serotonin in liver, kidney and brain tissues of rats.

Although the species differences in sensitivity of MAO towards the effect of inhibitory compounds are sometimes strikingly distinct[5, 12, 13] it is noteworthy that the rat liver MAO much more closely resembles the human liver mitochondrial MAO in this respect then the MAO from beef liver mitochondria.[5, 12, 13]

Although we have no experimental evidence for irreversibility of the inhibitory effect of the compounds studied, the use of 50 per cent homogenates of brain and liver tissues in our previous works[12, 13] with the well recognized reversible MAOI (harmine and α-methyltryptamine) decreased considerably the dissociation of enzyme-inhibitor complex and the inhibitory effects observed *in vivo* were quite demonstrable and comparable with the data obtained in other Laboratories.[14] These data and the published[14–16] characteristics of irreversibility of the inhibitory effects of MAOI studied lead us to conclude that the values indicating per cent of inhibition of deamination of the monoamines presented in this paper are essentially valid.

Pargyline has been chosen for this study as a clinically effective MAOI[14] exhibiting marked ability to cause selective inhibition of deamination of tyramine *in vitro*.[4, 6] The k_2 values expressing the velocities of interaction of pargyline with rat liver mitochondrial amine oxidases (or active sites belonging to a single macromolecule) deaminating tyramine and serotonin are $1{\cdot}5\text{–}3{\cdot}5 \times 10^5$ $1 \cdot mole^{-1}.min^{-1}$ and $1{\cdot}5\text{–}2{\cdot}5 \times 10^3$ $1 \cdot mole^{-1}.min^{-1}$, respectively.[6] Our present data demonstrate the selective inhibition by pargyline of tyramine deamination in all the tissues studied within 2 hr after a single subcutaneous injection of the drug into rats. Our data on the selectivity of the inhibitory effect of pargyline both *in vitro* and *in vivo* on deamination of tyramine are in complete agreement with recent results independently obtained in other Laboratories.[17, 18]

The selective effect of pargyline on deamination of various monoamines is so distinct that it may well be not only statistically but also biologically significant. Pargyline was found[19] to be more effective in prolongation and increasing the vaso-constricting effect of tyramine in rats as compared with iproniazid and marplan which according to the data of our previous experiments,[3, 4] contrary to pargyline are unable to exhibit any selectivity in blocking the oxidative deamination of tyramine as compared with serotonin. It could be expected that pargyline would readily cause accumulation of tyramine (and octopamine) at the postganglionic sympathetic nerve endings thus leading to substitution of noradrenaline for a "false neurotransmitter"[20] thus possibly explaining the well known effectiveness of pargyline as an antihypertensive drug.[21] Screening on the basis of the effect of pargyline derivatives on MAO activity by means of observation of appearance of a dark pigment due to oxidation of serotonin[22] although being undoubtedly a quick method may lead to erroneous results. Compounds selectively inhibiting deamination of tyramine and therefore being of interest as potential antihypertensive agents may be missed by workers using only this screening method.

Ability of phenelzine to cause an increase in content of serotonin in rat brain was observed,[23] but the alterations in content of phenylethylamine derivatives were not followed up in these experiments. Results of our *in vitro* experiments[4] with rat liver mitochondrial MAO demonstrate a certain selectivity in the inhibitory effect of phenelzine (nardil) on deamination of serotonin. *In vivo* we have noted a more distinct inhibition by phenelzine of serotonin deamination as compared with that of tyramine in liver but not in brain tissues. In kidney phenelzine inhibits deamination of tyramine to a higher degree as compared with that of serotonin.

It was shown that nialamid caused a significant (more than 3-fold) increase in content of serotonin in brain of mice while the content of dopamine remained practically unaltered.[24] These data are in agreement with our observations[3] on the selective inhibition by nialamide of deamination of serotonin catalyzed by rat liver mitochondrial MAO. However we could not demonstrate in rats any selectivity in the inhibitory effect of nialamide on oxidative deamination of biogenic monoamines *in vivo*.

Modaline—one of the new, non-hydrazine MAOI which attracted the considerable interest comparatively recently[16, 25]—does not inhibit MAO *in vitro*. *In vivo* it possesses a certain ability to cause statistically significant selective inhibition of deamination of tyramine as compared with that of serotonin in rat liver and, especially, in brain (but not in kidney) tissues (Table 1).

Compound Ro 4-2308 which is also inactive as MAOI *in vitro* was shown[26] to cause selective accumulation of dopamine in brain of mice. In our experiments it produced statistically significant selective inhibition of deamination of serotonin only in liver (but neither in brain nor in kidney) of rats.

The data obtained show the ability of certain MAOI to cause selective inhibition of enzymatic deamination of biogenic monoamines *in vivo*. Selective inhibition by pargyline of oxidative deamination of tyramine as compared with that of serotonin is especially distinct. Correlation between the results of studies *in vitro* and *in vivo* on selective inhibition by MAOI of enzymatic deamination of various monoamines was observed only in experiments with some inhibitors and in certain tissues. Assumptions on the occurrence of selective inhibition of deamination of various monoamines *in vivo* based on the results of *in vitro* studies have to be confirmed by direct experiments.

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