Biochemical Pharmacology, 1964, Vol. 13, pp. 1151–1156. Pergamon Press Ltd., Printed in Great Britain.

THE EFFECT OF DIETHYLTRYPTAMINE AND ITS DERIVATIVES ON MONOAMINE OXIDASE

Z. HUSZTI and J. BORSY

Research Institute for Pharmaceutical Industry,
Department of Pharmacology, Budapest, Hungary

(*Received* 8 *November* 1963; *accepted* 24 *March* 1964)

Abstract—The monoamine oxidase (MAO) inhibiting action of diethyltryptamine (DET) and its derivatives was studied with serotonin and tryptamine as substrates. A marked difference was found between the inhibitory power of DET and of the DET analogues and also in the types of inhibition. A significant change in the degree of inhibition was observed after the serotonin had been replaced by tryptamine. Zeller's recent enzyme-complex hypothesis was used to explain the discrepancies in inhibitory power called forth by a slight change in the structure of the inhibitor or that of the substrate.

A NUMBER of authors[1–5] have studied the biological role and pharmacological activity of the 3-2-(dialkyaminoethyl) indoles, including DET.* Govier[6] was the first to observe the biochemical action of DET. He has found that DET, in contrast to tryptamine is not oxidised by guinea-pig liver homogenate, but it inhibits the tyramine oxidation. By using in an *in vitro* pharmacological test Vane[7] has also demonstrated, that DET is not metabolized by rat fundus amine oxidase. The MAO inhibiting action of DET was studied by Satory *et al.*[8] According to their results DET inhibits the MAO activity of rat brain both *in vitro* and *in vivo*. According to Szara's classification[9] DET belongs to that group of tryptamine derivatives which raises the serotonin concentration of certain parts of the brain.

It seems interesting to investigate the MAO inhibiting action of DET derivatives substituted in the 2-position and to compare their inhibiting action with that of the DET.

MATERIALS AND METHODS

For enzyme activity measurements serotonin creatinin sulphate (Leclerc) and tryptamine HCl (Fluka), analytical grade, were used. DET and its derivatives were synthetized in our Institute by the method of Lénard.[10] The DET was obtained as a hydrochloride, the other derivatives as ethane sulphonate compounds. Liver and brain homogenates of white rats in 2 : 1 proportion of water : wet tissue were used as enzyme preparation. MAO activity was assayed both according to Creasey's method[11] by measuring the oxygen consumption and according to Zile's method by determining the serotonin consumption.

The composition of the Creasey's mixture was: serotonin 10 μmoles or tryptamine 20 μmoles, semicarbazide 30 μmoles, potassium cyanide 30 μmoles, sodium phosphate

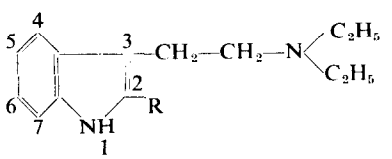
* Abbreviations used: DET-diethyltryptamine, MAO-monoamine oxidase.

buffer 50 μmoles, pH 7·4. The inhibitors studied were used in a concentration range of 0·3–30 μmoles. 330 mg freshly homogenized liver tissue were used as enzyme preparation in each assay mixture. The mixture was made up with distilled water to a total volume of 3 ml.

The composition of the Zile's mixture was: serotonin 1 to 10 μmoles, sodium phosphate buffer 130 μmoles, pH 7·4, inhibitors 0·3–30 μmoles and 100 mg freshly homogenized liver tissue, or 230 mg freshly homogenized brain tissue, in a total volume of 3 ml. Mixtures were preincubated for 25 min at 37° in a shaking incubator. The reaction was started by addition of the substrates and was stopped after 30 min incubation by adding 5 ml 5% trichloroacetic acid solution. Mixtures containing no inhibitor were used as controls.

For the study of the MAO activity *in vivo* white male rats (120–160 g) were used. The animals were injected i.p. with doses of 30 mg/kg of DET or DET derivatives, and were killed 15, 30 or 60 min later by decapitation. The rat brains were homogenized and the MAO activity of the homogenates was determined by Zile's method. For all experiments 4–5 untreated animals were used as controls.

TABLE 1. *In vitro* INHIBITION OF MONOAMINE OXIDASE BY DET AND ITS DERIVATIVES



Ring substituent R	Trivial name used in text Chemical name	I_{50} Serotonin	I_{50} Tryptamine
———	DET 3-(2-diethylaminoethyl)indole	$5{\cdot}9 \times 10^{-5}$ M	5×10^{-3} M
phenyl	phenyl-DET 2-(phenyl-3(2-diethylamino-ethyl)indole	$1{\cdot}1 \times 10^{-3}$ M	$2{\cdot}2 \times 10^{-3}$ M
diphenyl	diphenyl-DET 2-(diphenyl)-3(2-diethylamino-ethyl)indole	$1{\cdot}4 \times 10^{-3}$ M	$2{\cdot}4 \times 10^{-3}$ M
4-methylphenyl	4-methylphenyl-DET 2-(4-methylphenyl)-3(2-diethyl-aminoethyl)indole	$1{\cdot}5 \times 10^{-3}$ M	$3{\cdot}2 \times 10^{-3}$ M
naphtyl	naphtyl-DET 2-(naphtyl)-3(2-diethylamino ethyl)indole	$2{\cdot}1 \times 10^{-3}$ M	$2{\cdot}1 \times 10^{-3}$ M
4-chlorphenyl	4-chlorphenyl-DET 2-(4-chlorphenyl)-3(2-diethyl-aminoethyl)indole	$2{\cdot}6 \times 10^{-3}$ M	$2{\cdot}8 \times 10^{-3}$ M
4-methoxyphenyl	4-methoxyphenyl-DET 2-(4-methoxyphenyl)-3(2-diethyl-aminoethyl)indole	4×10^{-3} M	$1{\cdot}3 \times 10^{-3}$ M
3,4-dimethoxyphenyl	3,4-dimethoxyphenyl-DET 2-(3,4-dimethoxyphenyl)-3(2-diethylaminoethyl)indole	6×10^{-3} M	$2{\cdot}4 \times 10^{-3}$ M

Inhibitions were calculated using as control values Q_{ox}: 22–24 for serotonin and Q_{ox}: 19–21 for tryptamine.

Q_{ox} values calculated from the initial portion of the curve of oxygen consumption are defined as a number of micromoles of oxygen consumed/hr per g fresh tissue. The Q_{ser} values represent the consumption of serotonin in μmoles/hr per g fresh tissue.

I_{50} is the concentration of the inhibitor required to produce 50% inhibition of the initial oxygen uptake. The I_{50} values were determined graphically, the percentages of MAO inhibition were plotted against the logarithms of the inhibitor concentration.

RESULTS

The I_{50} values determined in the presence of different inhibitors by the above methods are summarized in Table 1.

The inhibitory effects of DET and phenyl-DET as a function of the log concentrations are shown in Figs. 1 and 2. No linear correlation was found between the inhibitory effect of DET and its log concentration on tryptamine oxidation.

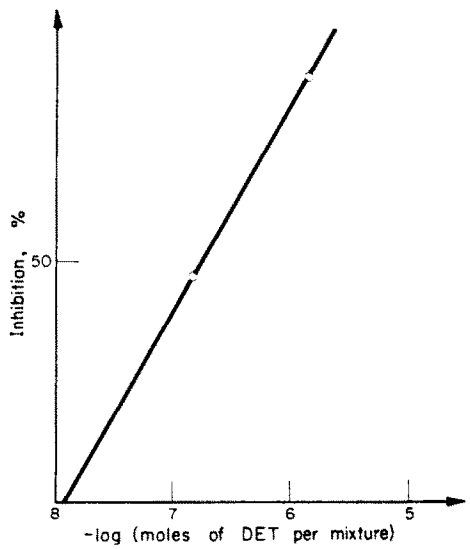


FIG. 1. Relationship between the concentration and the inhibitory effect of DET in the presence of serotonin as substrate.
DET concentrations are expressed as − log moles of DET/3 ml.

The effect of substrates on the inhibition of MAO by DET and phenyl-DET was determined by both Creasey's and Zile's procedure. As is shown in Table 2 the extent of inhibition is not influenced by the quantity of substrate used.

Table 3 shows the effect of dialysis. The liver homogenate was incubated with the inhibitor for 25 min and the reaction mixture dialysed for 24 hr at 5° against a 10^{-3} M phosphate buffer, pH 7·4. MAO activity was measured after addition of 1 μmole of serotonin. Reaction mixture without inhibitor was used as a blank. The inhibition caused by phenyl-DET was not influenced by the dialysis, while no MAO inhibition was observed by using DET as inhibitor, after the dialysis.

Figure 3 shows the inhibitory action of phenyl-DET as a function of the preincubation time. Between 0 and 15 min, inhibition increases, thereafter no further change was observed in the inhibition.

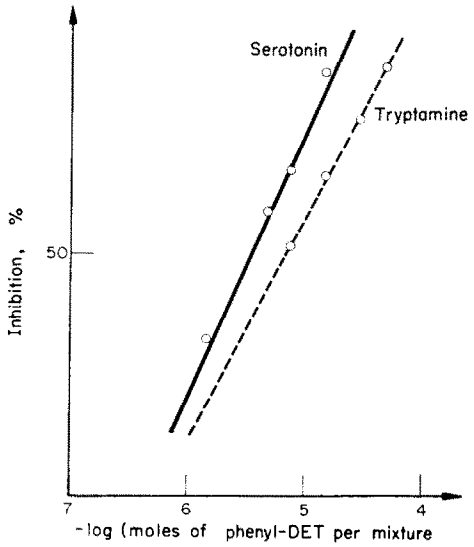


FIG. 2. Relationship between the concentration and the inhibitory effect of phenyl-DET. Phenyl-DET concentrations are expressed as — log moles of phenyl-DET/3 ml.

TABLE 2. THE EFFECT OF SUBSTRATE CONCENTRATION ON THE MAO INHIBITION PRODUCED BY DET AND PHENYL-DET.

Final concentration of serotonin	Controls				Inhibitions (%)		
	Serotonin		Oxygen		DET		phenyl-DET
	P	Q_{ser}	P	Q_{ox}	5×10^{-4} M	5×10^{-5} M	$2{\cdot}5 \times 10^{-3}$ M
$3{\cdot}3 \times 10^{-4}$ M	10	17·4			83	57	
$3{\cdot}3 \times 10^{-3}$ M	100	18·4	30	22·7	83	54	65
$6{\cdot}6 \times 10^{-3}$ M	200	18·6	60	23·0	80	52	59
$1{\cdot}3 \times 10^{-2}$ M			120	22·2			67

P values are defined as the number of μ moles of substrate /g fresh tissue. Values of percentages are the average of two or four measurements.

TABLE 3. THE EFFECT OF DIALYSIS ON THE MAO INHIBITION PRODUCED BY DET AND PHENYL-DET.

Time of dialysis (hr)	Controls		Inhibition (%)				
	I	II	DET I			Phenyl-DET II	
	Q_{ser}	Q_{ser}	5×10^{-3} M	5×10^{-4} M	5×10^{-5} M	10^{-2} M	10^{-3} M
0	17·2	17·4	100	83	57	100	47
24	10*	13·2	0	0	0	100	45

* After the dialysis the enzymatic activity of the homogenates decreased.

Thirty mg/kg dose of DET i.p. produces *in vivo* a 67% inhibition of MAO activity of the brain. Fifteen min after injection, the inhibitory effect of DET decreases. The inhibitory effect of all DET compounds studied in the present paper is less pronounced than that of the unsubstituted compound.

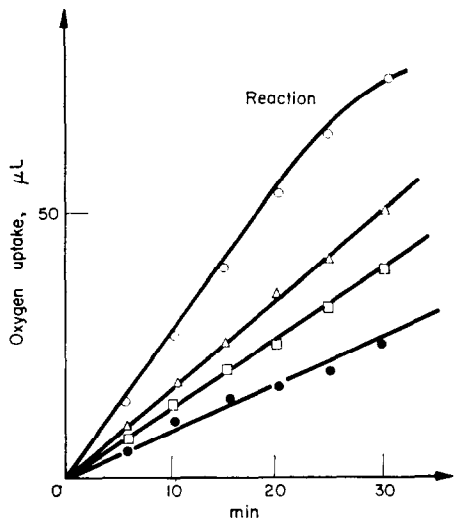


FIG. 3. Effect of length of the preincubation on inhibition caused by phenyl-DET.
○ without inhibitor, △, □, ● curves after 5, 10 and 15 min preincubation with $2{\cdot}5 \times 10^{-3}$ M of phenyl-DET. $3{\cdot}3 \times 10^{-3}$ M of serotonin was used as substrate and 1 ml of 2 : 1 rat liver homogenate as enzyme preparation.

DISCUSSION

In the presence of serotonin substrate the inhibiting effect of DET derivatives on MAO is about 100 times lower than that of DET. 4-methoxyphenyl-DET and 3,4-dimethoxyphenyl-DET possess the lowest inhibitory effect. There is also a difference in the type of inhibition caused by DET and its derivatives. The inhibitory action of DET is reversible, while that of the phenyl-DET is irreversible. In both cases the inhibition is independent of the concentration of the substrate.

It seems remarkable that the inhibitory effects detected by the assay of tryptamine as substrate differ from those measured on serotonin. The change of serotonin to tryptamine results in a hundred fold decrease of the inhibitory action of DET. When tryptamine was used as substrate the inhibitory effect of phenyl-DET, diphenyl-DET, 4-methylphenyl-DET was about half of the value measured with serotonin. Naphthyl-DET and 4-chlorophenyl-DET inhibit the MAO activity in the presence of both tryptamine and serotonin at about the same extent. 4-methoxyphenyl-DET and 3,4-dimethoxyphenyl-DET, however, cause a more pronounced inhibition when tryptamine was used as substrate.

Barlow[13] suggests for the explanation of the dependence of the inhibitory action of dimethyl-tryptamine and its 2-methyl derivative on the type of the substrate, that different enzymes might eventually participate in the oxidation of tryptamine and serotonin respectively, these enzymes being closely related but with somewhat of a difference in the geometry of the bond surface. Since we found no compound which would inhibit the oxidation of serotonin only, or tryptamine only, we believe that

Zeller's recent eutope and dystope complex theory[14] might be more suitable to provide an answer to the problem. Zeller explains the change in the inhibitory value when replacing a substrate by another one with nearly identical structure, with a change in the orientation of the substrate-enzyme attachment for which certain steric factors might be responsible. This theory might explain the lower inhibitory effect of 4-methoxyphenyl-DET and 3,4-dimethoxyphenyl-DET on serotonin. For this variation the earlier assumption concerning that the relative affinity of MAO is greater for tryptamine than that for serotonin,[15] offers no explanation.

In contrast to DET, derivatives studied in the present paper display no inhibitory effect *in vivo*.

Acknowledgement—The authors wish to thank Miss Maria Küronya and Mrs. Eva Máriássy for the competent technical assistance.

REFERENCES

1. V. ERSPAMER, *Pharmacol. Rev.* **6**, 425 (1954).
2. ST. SZÁRA, *Experientia (Basel)* **12**, 411 (1956).
3. R. B. BARLOW and I. KHAN, *Brit. J. Pharmacol.* **14**, 99 (1956).
4. J. BORSY, Thesis, Budapest (1960).
5. A. K. PFEIFER, É. SÁTORY and I. PATAKY, *Acta Physiol. Acad. Sci. Hung.* **19**, 225 (1961).
6. W. M. GOVIER, B. G. HOWES and A. J. GIBBONS, *Science*, **118**, 596 (1953).
7. J. R. VANE, *Brit. J. Pharmacol.* **14**, 87 (1959).
8. É. SÁTORY, A. K. PFEIFER, L. KEREKES and I. PATAKY, 26 Kongress der Ungarischen Physiologischen Gesellschaft Debrecen (1960).
9. ST. SZÁRA, Abstracts of IIIrd Congress of CINP pp. 100 (1962).
10. K. LÉNARD and J. BORSY, *Acta Chim. Acad. Sci. Hung.* **34**, 439 (1962).
11. N. H. CREASEY, *Biochem. J.* **64**, 178 (1956).
12. M. ZILE and H. A. LARDY, *Arch. Biochem. Biophys.* **82**, 411 (1959).
13. R. B. BARLOW, *Brit. J. Pharmacol.* **16**, 153 (1961).
14. E. A. ZELLER, *Ann. N.Y. Acad. Sci.* **107**, 811 (1963).
15. E. A. ZELLER, *J. Neuropsychiatry*, Suppl. **1**, 125 (1961).