Biochemical Pharmacology, Vol. 23, pp. 1138–1140. Pergamon Press, 1974. Printed in Great Britain

Inhibition of lung, liver and brain monoamine oxidase by imipramine and desipramine*

(*Received* 26 *April* 1973; *accepted* 22 *August* 1973)

THE BIOGENIC amines, 5-hydroxytryptamine (5-HT) and norepinephrine (NE), may be significant determinants of behavior in the depressed state.[1] Tricyclic antidepressant drugs are believed to function by blocking neuronal and synaptosomal binding of catechol and indole amines in the brain, thus increasing the monoamine content of the tissue.[2,3] However, several recent studies have demonstrated a lack of correlation between the ability of these tricyclic drugs to prevent uptake and binding of biogenic amines in the brain and clinical efficacy of these drugs.[4–6] Several papers also have yielded data consistant with inhibition of monoamine oxidase (MAO) by imipramine. Thus, Bruinvels[7] found that imipramine not only increases brain 5-HT but also decreases the 5-HT deaminated product, 5-hydroxyindole acetic acid (5-HIAA). A similar decrease in 5-HIAA accumulation in the brain after administration of chlorimipramine has been reported by Meek and Werdinius.[8] Imipramine and desipramine have also been shown to decrease NE-deaminated products in the brain.[9,10] Although the results of these papers could reflect imipramine inhibition of MAO, in each case alternate conclusions were drawn from the data. In this paper we will present evidence which suggests that tricyclic antidepressant drugs, imipramine and desipramine, are effective inhibitors of MAO *in vitro*.

The lungs, livers and brains from male albino rabbits were each minced in two vol. of 0·1 M potassium phosphate buffer. Lungs and livers were homogenized two times (3 sec each time) in a Waring Blender and then in a motor driven Teflon-glass homogenizer. Minced brains were homogenized only in the Teflon-glass homogenizer. Lung homogenates were further diluted by addition of one vol. of buffer. The final homogenates were centrifuged at 600 *g* for 10 min to remove cellular debris and the supernatant solutions, containing MAO, were stored at −20°. Protein concentrations were determined by the biuret method.[11]

Reaction flasks contained 0·05 m-mole of potassium phosphate buffer, pH 7·4, either 2·2 nmoles ^{14}C-5-HT or 2·3 nmoles ^{14}C-NE, varying amounts of protein and varying amounts of desipramine or imipramine in a total volume of 2 ml. Tropolone (2×10^{-4} M final concentration), to inhibit catechol-*O*-methyl transferase, was added to the flasks containing NE. The incubations were terminated by the addition of 2 ml of 0·4 M perchloric acid and the precipitated protein was removed by centrifugation at 3000 *g* for 10 min.

Three milliliters of the resulting supernatant solutions (adjusted to pH 6) was passed over Bio Rex-70 (100–200 mesh, sodium form, pH 6·0) columns (approximately $3 \times 0{\cdot}7$ cm), which then were washed with H_2O to a total eluate volume of 8 ml. The aldehyde and acid products formed in the reactions were eluted in the 8 ml of eluate, whereas amine-starting materials remained bound to the cation resin. A similar method using Bio Rex-70 columns to determine brain catecholamine and indoleamine levels has been described previously.[12] Aliquots (0·5 ml) of the eluates were added to 10 ml of Bray's[13] solution and the radioactivity was determined in a Packard Tri-Carb scintillation spectrometer (model 3320). In some initial studies 0·25 M HCl was used to elute the amines from the columns. Total recovery of radioactivity from the columns was at least 90 per cent.

The effects of desipramine on the ability of lung homogenates to deaminate 5-HT and NE are illustrated in Fig. 1. Desipramine at a concentration of approximately 3×10^{-4} M inhibits MAO activity 50 per cent. Less than 10 per cent inhibition is observed at desipramine concentrations below 5×10^{-5} M and almost complete inhibition, greater than 85 per cent, is observed at concentrations greater than 10^{-3} M. Also it was found that as protein concentration of the crude enzyme preparations was decreased in the incubation medium, the percentage inhibition of 5-HT deamination increased. Desipramine also effectively inhibits deamination of other biogenic amines, epinephrine, tyramine and dopamine. As with 5-HT and NE, only a slight decrease in deamination of all three substrates was observed in the presence of 5×10^{-5} M desipramine, and approximately 50 per cent inhibition was observed in the presence of 5×10^{-4} M.

To insure that lung MAO was not unique in its susceptibility to inhibition by desipramine, similar experiments were performed with liver and brain preparations of MAO. As shown in Table 1, deamination of 5-HT by either liver or brain homogenates also was inhibited by desipramine. At a concentration of 5×10^{-4} M desipramine, approximately 40 per cent of MAO activity was inhibited and at 5×10^{-5} M or below, only a slight decrease in liver and brain MAO activity was observed. The inhibition by desipramine of 5-HT deamination with lung, liver and brain preparations of MAO appeared to be of a mixed type based on Lineweaver–Burk plots.

* This investigation was supported by Public Health Service Grant no. 13315 from the National Heart and Lung Institute and no. 435 from the Connecticut Heart Association. Assistance was also provided by Ayerst Laboratories and Lilly Research Laboratories.

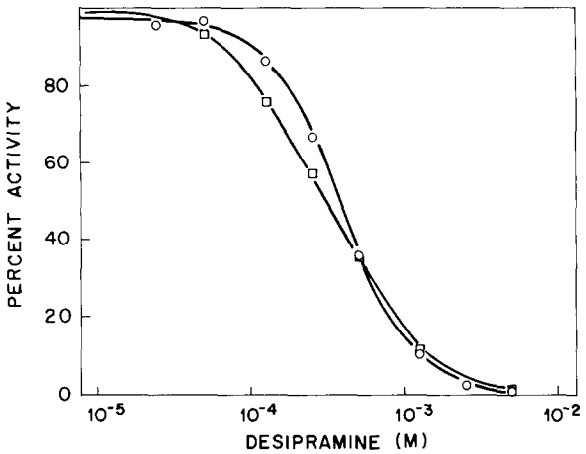

FIG. 1. Effect of desipramine on the deamination of 5-HT (○—○) and NE (□—□) by lung MAO. Reactions were initiated with the addition of lung 600 *g* supernatant solution. Final protein concentration was 4·2 mg protein/ml. Reaction flasks containing 5-HT and NE were incubated for 15 and 40 min respectively.

The ability of imipramine, the *N*-methyl derivative of desipramine, to inhibit deamination of 5-HT with lung, liver and brain preparation of MAO also was determined. The results of this experiment, shown in Table 2, indicate that imipramine is also an effective inhibitor of MAO. At a concentration of 5×10^{-5} M imipramine, only slight inhibition of 5-HT metabolism is observed whereas at a concentration of 5×10^{-4} M, MAO activity is decreased approximately 40–60 per cent.

The data in this paper show the ability of the tricyclic antidepressant drugs, imipramine and desipramine, to inhibit deamination of 5-HT, NE and other biogenic monoamines *in vitro*. The fact that desipramine also decreases metabolism of the secondary amine, epinephrine, establishes that these antidepressant drugs are inhibiting the enzyme, monoamine oxidase.[14]

Dingell *et al.*[15] have shown that imipramine accumulates to the greatest extent in rabbit lung (61·8 μg/g) after two i.p. injections of the tricyclic antidepressant drug, given 15 min apart. Accumulation in brain and liver amounted to about one-fifth and one-tenth, respectively, of that in lung. It is difficult to assess our data in terms of tissue concentration of imipramine and ability of tricyclic antidepressant drugs to inhibit MAO, *in vitro*, since the values for per cent inhibition obtained with crude enzyme preparations herein most likely underestimate the true value. This results from imipramine binding nonspecifically to excess protein present in the crude enzyme preparations, thus lowering the imipramine concentration which can effectively inhibit MAO.

The results of this paper, however, are in agreement with a previous report indicating that deamination of kynuramine using highly purified preparations of rabbit and pig liver mitochondrial MAO was inhibited by imipramine.[16] The concentration of imipramine needed to inhibit the reaction by 50 per cent, $1·6 \times 10^{-4}$ M, is similar to the desipramine concentration, 3×10^{-4} M, obtained herein for the inhibition of 5-HT and NE deamination. The authors of the latter paper also noted a correlation between the I_{50} of several MAO inhibitors, including imipramine, and the average daily clinical dose of the drugs.

TABLE 1. DESIPRAMINE INHIBITION OF LIVER AND BRAIN MONOAMINE OXIDASE*

Desipramine conen (M)	nmoles 5-HIAA formed				Average % inhibition	
	Liver		Brain			
	1	2	1	2	Liver	Brain
None	0·237	0·296	0·608	0·511		
1×10^{-5}	0·232	0·298	0·618		1·1	0
5×10^{-5}	0·214	0·286	0·587		6·6	3·5
5×10^{-4}	0·139	0·173	0·380	0·347	41·5	34·8

* Reactions were initiated with the addition of liver or brain 600 *g* supernatant solution. Final protein concentration was 11·0 and 11·8 mg protein/ml respectively. Reactions were incubated for 15 min at 37

TABLE 2. IMIPRAMINE INHIBITION OF LUNG, LIVER AND BRAIN MONOAMINE OXIDASE*

Imipramine conen (M)	Lung	Liver	Brain	% Inhibition		
	(nmoles 5-HIAA formed)			Lung	Liver	Brain
None	0·604	0·237	0·608			
1×10^{-5}	0·598	0·224	0·604	1·0	5·5	0·7
5×10^{-5}	0·518	0·226	0·600	14·2	4·6	1·3
5×10^{-4}	0·241	0·130	0·372	60·0	45·2	38·8

* Reactions were initiated with the addition of lung, liver or brain 600 *g* supernatant solution. Final protein concentration was 4·2, 11·0 and 11·8 mg protein/ml respectively. Reactions were incubated for 15 min at 37°.

The clinical relevance of the findings presented in this paper remains to be determined. However our data raise the possibility that tricyclic antidepressant drugs may act in man not only by preventing uptake of biogenic amines by brain neurones but may also prevent loss of these monoamines by inhibiting monoamine oxidase.

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Biochemical Pharmacology, Vol. 23, pp. 1140–1143. Pergamon Press, 1974. Printed in Great Britain.

Blood and brain levels of Δ^1-tetrahydrocannabinol in mice
The effect of 7-hydroxy-Δ^1-tetrahydrocannabinol

(*Received* 15 *August* 1973; *accepted* 4 *October* 1973)

IN A PREVIOUS paper,[1] it was reported that after intravenous injection of ^{3}H-7-hydroxy-Δ^1- tetrahydrocannabinol (^{3}H-7-hydroxy-Δ^1-THC) into mice, a non-polar component was found in the blood extract, but to a much lesser extent in the brain extract. Both thin-layer and gas liquid chromatography indicated