Effects of Psychotropic Compounds on Enzyme Systems, II. *In vitro* Inhibition of Monoamine Oxidase.* (26965)

EARL USDIN AND VERA R. USDIN
Institute of Scientific Research, New Mexico Highlands University, Las Vegas, N. Mex.

Our objective of attempting to correlate psychotropic action with effects on enzyme systems has been described(1). The significance of monoamine oxidase (MAO) and MAO inhibitors in relation to psychotropic compounds has been discussed by Zeller(2). We have adopted the method of Weissbach *et al.*(3) for determination of MAO, in order to obtain the necessary data for determination of K_i values(4).

Materials and methods. Kynuramine dihydrobromide was obtained from the Regis Chemical Co.; psychotropic compounds were supplied by the manufacturers, as listed in Table I. Livers of normal adult albino rats were homogenized in ice water and lyophilized. The lyophilized powder was stored in the refrigerator and served as the source of enzyme. No loss in activity was observed during storage for 3 months. The liver suspension was prepared fresh each day by homogenizing 250 mg of the powder in 6 ml of cold deionized water. This solution was kept at 0° throughout the day. Kynuramine dihydrobromide, 10 mg, was dissolved in 100 ml of H_2O. This solution was stored in the refrigerator. The compounds being tested were first prepared by dissolving 10 mg either in 25 ml of water or in 2 ml of ethanol and then adding water to the 25 ml mark. In the case of insoluble free bases, 0.1 ml of concentrated HCl was added to effect solution. After the preliminary run at an inhibitor concentration of 10 mg per 25 ml, the kinetic studies were made at such a concentration that significant differences were obtained among the tubes containing no inhibitors and those containing various amounts of inhibitor. To control pH, a 0.5 M phosphate buffer of pH 7.4 was used.

To each of twenty-two 18 × 150 mm Pyrex culture tubes, 0.1 ml of the liver suspension was added. A sufficient quantity of deionized water was added so that the final quantity of liquid in the tube was 6.0 ml. 0.3 ml of the phosphate buffer was placed into each tube. No inhibitor was added to the first 6 tubes, 0.5 ml to the next 5 tubes, 1.0 ml to the next 5 and 1.5 ml to the final 5 tubes. All tubes were thoroughly mixed at this point and the timer started. The first tube was used as a blank, therefore no kynuramine was added to it. To the first tube in each series (tubes #2, 7, 12 and 17), 0.5 ml of the kynuramine solution was added; 0.7 ml to the second tube; 1.0 ml to the third; 1.5 ml in the fourth and 2.0 ml in the last tube of each series. A tube containing all of the components except kynuramine, but including the highest level of inhibitor, was run to determine the contribution of the inhibitor to the observed optical density. After being thoroughly mixed, the tubes were incubated in a 37°C bath. They were read at 360 mμ in a Bausch and Lomb Spectronic 20 against the first tube as a blank after 5, 10, 20 and 30 minutes. All runs were made in duplicate.

After subtracting the contribution of inhibitor from each observed O. D., the O. D.s were adjusted, using a standard table(5). When these values were plotted, the velocity of the reaction (=V) was obtained from the slope. When Lineweaver-Burk plots(4) of 1/V vs. 1/S were made, the lines for each inhibitor concentration intercepted that for zero inhibitor concentration at the point for which $1/S = 0$. This would indicate competitive inhibition. To obtain K_i, the value for the quotient of the slope of the line at a given inhibitor concentration divided by the slope of the line with no inhibitor was plotted vs. the inhibitor concentration. The inhibitor concentration corresponding to a quotient of 2.00 was equal to K_i. For plotting the straight lines, a method of least squares was used.

* Supported in part by grants from Nat. Inst. of Mental Health and New Mexico Heart Assn.

TABLE I. K_i Values for Inhibition of MAO by Selected Psychotropic and Related Compounds

Compound	Action	Mfr.	K_i (M)	Compound	Action	Mfr.	K_i (M)
Acepromazine	T	CB	No eff.	Megimide	E	A	No eff.
Adrenochrome	H	AH	1.8×10^{-1}	Meperidide	T	J	3.9×10^{-6}
AF-365	T	AF	3.5×10^{-1}	Mer 16		MER	2.2×10^{-5}
Amitriptyline	E	M	2.8×10^{-5}	Methamphetamine	E	A,BW	9.8×10^{-5}
Anhalamine	H	P	No eff.	Methonalide	T	BR	No eff.
Anisoperidone	T	J	1.1×10^{-5}	Methopromazine	T	LE	6.6×10^{-5}
Arecoline		P	2.0×10^{-3}	Methylphenidate	E	CI	4.5×10^{-4}
Benactyzine	T	M,MD	5.9×10^{-1}	α-methyltryptamine		U	2.1×10^{-4}
Benztropine methane sulfonate		M	1.6×10^{-5}	Nialamide	E	PF	5.8×10^{-6}
BOL-148		S	2.7×10^{-4}	NP-207	T	S	8.4×10^{-5}
Butropipazone		J	3.7×10^{-5}	O.C. 1131-1417	T	M	5.2×10^{-5}
BW 58-271	T	BW	4.2×10^{-4}	Olympax		D	1.4×10^{-4}
CB-1497		CB	5.6×10^{-5}	Orphenadrine	E	R	2.2×10^{-5}
CB-1519		CB	8.5×10^{-5}	Oxanamide	T	MER	No eff.
CB-1613		CB	9.5×10^{-5}	Pentylenetetrazol	E	K	1.2×10^{-3}
CB-1619		CB	3.8×10^{-5}	Petrichloral		IC	No eff.
CB-1620		CB	7.9×10^{-5}	Phenaglycodol	T	LI	4.4×10^{-4}
CB-1658		CB	4.7×10^{-5}	Phencyclidine	H	PD	1.5×10^{-4}
Chlophedianol		R	5.6×10^{-5}	Phendimetrazine	E	AY	No eff.
Chlordiazepoxide	T	HR	No eff.	Phenmetrazine	E	G	4.6×10^{-4}
Chlormethazanone	T	WI	No eff.	Phenyltoxamine	E	BR	2.0×10^{-4}
Chloroxazone		MC	2.7×10^{-4}	Pipamazine	T	SE	No eff.
Chlorpromazine	T	SKF	9.9×10^{-5}	Piperazine pentanol		PD	2.9×10^{-4}
Chlorprothixen	T	HR	3.0×10^{-5}	Piperidine phosphate		A	5.2×10^{-4}
Chlortrimeton		SC	2.4×10^{-4}	Pipradrol	E	MER	5.8×10^{-4}
CI-383	T	PD	3.8×10^{-4}	Prochlorperazine	T	SKF	1.2×10^{-4}
CI-384	T	PD	2.7×10^{-4}	Profenamine	T	RP	5.8×10^{-4}
CI-400	H	PD	3.6×10^{-4}	Promethazine	T	WY	1.5×10^{-4}
Cqd-280		PH	4.7×10^{-4}	Propiomazine	T	WY	9.7×10^{-5}
Cqd-285		PH	8.4×10^{-4}	Prothipendyl HCl	T	AY	6.5×10^{-5}
Cqdd-280		PH	2.4×10^{-4}	Psilocybin	H	S	No eff.
Csat-131		PH	2.4×10^{-4}	Pyrrolazote	T	U	8.1×10^{-5}
Cyclohexalamine		PD	No eff.	Rec 7-0268	E	REC	No eff.
Cyproheptadine		M	3.9×10^{-5}	Rescinnamine	T	PF	"
Deanol	E	R	No eff.	Reserpine	T	CI	Insol.
Diethazine	T	RP	2.5×10^{-4}	Riker 566	E	R	No eff.
Diethylpropion		MER	1.5×10^{-3}	Riker 594		R	"
Diphenylhydantoin	T	PD	No eff.	RO 2-6797	E	HR	3.2×10^{-5}
Diphenhydramine		PD	9.7×10^{-4}	RO 4-1027	E	HR	2.5×10^{-5}
Dyclonine		PM	2.3×10^{-6}	RO 4-1038		HR	2.8×10^{-5}
Ectylurea	T	A,U	No eff.	RO 4-1340		HR	5.8×10^{-5}
Ethychlorvynol	T	A	1.1×10^{-4}	RO 4-1385	E	HR	3.5×10^{-5}
Fluphenazine	T	SQ,WH	1.1×10^{-4}	RO 5-0994	E	HR	4.6×10^{-5}
Haloperidide	T	J	2.3×10^{-6}	RO 5-1162		HR	4.5×10^{-6}
Hexacyclonate		WL	No eff.	RO 5-1221		HR	1.1×10^{-5}
Hydroxyphenamate		AR	1.5×10^{-4}	S 9-888		PF	1.0×10^{-4}
Hydroxyzine	T	PF	2.8×10^{-5}	SA-97		A	2.4×10^{-5}
Imipramine	E	G	3.0×10^{-5}	SC-10600	E	SE	No eff.
IN-33	E	IN	No eff.	Styramate		AR	3.4×10^{-4}
IN-399	T	IN	1.3×10^{-5}	Tersavid		HR	3.1×10^{-5}
Iproniazid	E	HR	1.2×10^{-5}	Tetrabenazine	T	HR	1.3×10^{-4}
Isocarboxazide	E	HR	8.5×10^{-7}	Thiopropazate	T	SE	1.2×10^{-4}
Isopyrin		BG	No eff.	Thioproperazine	T	RP	2.2×10^{-4}
JB-318	H	LA	1.6×10^{-4}	Thymoxyalkylamine		DI	3.9×10^{-4}
JB-336	E	LA	4.6×10^{-4}	Tricylamol		BW	2.5×10^{-5}
JB-835		LA	3.9×10^{-6}	Trifluoperazine	T	SKF	6.9×10^{-5}
KS-24		S	6.4×10^{-5}	Trimeprazine	T	SKF	1.4×10^{-4}
KS-33		S	1.1×10^{-4}	Triperidol	T	J	4.8×10^{-5}
KS-75		S	5.6×10^{-4}	Win 2299		WI	4.0×10^{-4}
Levomepromazine	T	LE	2.1×10^{-4}	WV 357	T	DI	5.9×10^{-5}
Lidepran	E	RP	1.5×10^{-3}	WV 760	T	DI	3.7×10^{-4}
Lysergic acid diethylamide	H	S	No eff.	WV 770	T	DI	6.5×10^{-6}

ABBREVIATIONS:

E = energizer; H = hallucinogen; T = tranquilizer

A = Abbott Labs.; AF = Angelini Francesco; AH = A. Hoffer; AR = Armour Pharmaceutical Co.; AY = Ayerst Labs.; BR = Bristol Labs.; BW = Burroughs Wellcome and Co.; BG = Byk-Gulden; CB = Clin-Byla; CI = Ciba; D = Laboratoires Dausse; DI = Diwag Chemische Fabriken; G = Geigy Research Labs.; HR = Hoffmann-La Roche Inc.; IC = Ives-Cameron Co.; IN = Irwin, Neisler & Co.; J = Research Laboratorium Dr. C. Janssen; K = Knoll Pharmaceutical Co.; LA = Lakeside Labs.; LE = Lederle Labs.; LI = Eli Lilly & Co.; M = Merck Inst.; MC = McNeil Labs.; MD = Meco-Dumex; MER = Wm. S. Merrell Co.; P = S. B. Penick & Co.; PD = Parke, Davis & Co.; PF = Chas. Pfizer & Co.; PH = Philips-Duphar; PM = Pitman-Moore Co.; R = Riker Labs.; REC = Recordati Lab. Farmacol.; RP = Rhone-Poulenc; S = Sandoz Pharmaceuticals; SC = Schering Corp.; SE = G. D. Searle & Co.; SKF = Smith Kline & French Labs.; SQ = Squibb Inst. for Medical Research; U = Upjohn Co.; WH = White Laboratories; WI = Winthrop Labs.; WL = Warner-Lambert Research Inst.; WY = Wyeth Labs.

Results and discussion. Table I † lists K_i values obtained for a number of compounds.

Almost all of the compounds which were inhibitory exhibited competitive inhibition, at least at concentrations close to K_i. When some of these compounds were run at higher concentrations, the values could not be fitted to a straight line. However, this does not interfere with the calculation of K_i nor with the kinetic studies at the lower concentrations.

No obvious relationships between psychotropic action and MAO inhibition can be discerned from an examination of the data. Some of the compounds which were potent MAO inhibitors had tranquilizing properties while others acted as energizers; among the compounds with no MAO-inhibiting effects were tranquilizers, energizers and hallucinogens. Within a chemical series, however, there was some degree of correlation between potency and MAO K_i values. Thus, if one makes a table of phenothiazines arranged in order of K_i values and adds suggested dose levels, the compounds with lower K_i also have lower dose amounts (Table II).

TABLE II. MAO Inhibition and Dose Levels for Some Phenothiazines.

Phenothiazine	K_i (M)	Dose (mg)
Trifluoperazine	6.9×10^{-5}	4
Fluphenazine	1.1×10^{-4}	5
Prochlorperazine	1.2×10^{-4}	15
Thiopropazate	1.2×10^{-4}	15
Levomepromazine	2.2×10^{-4}	20-40
Diethazine	2.5×10^{-4}	20-50
Profenamine	5.8×10^{-4}	20-50
Chlorpromazine	9.9×10^{-3}	10-100
Acepromazine	No effect	150-200

† A table listing structural formulae and other properties of these compounds and of other psychotropic compounds may be obtained from the authors.

Summary. K_i values for inhibition of MAO *in vitro* by 122 psychotropic compounds have been determined. At inhibitor concentrations close to or below the K_i value, the inhibition was competitive. MAO-inhibitors were found among tranquilizers, energizers and hallucinogens. The only correlation between MAO inhibition and psychotropic effects that could be found was within a chemical series.

1. Usdin, V. R., Su, Su-Chen, Usdin, E., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 457.

2. Zeller, E. A., *J. Neuropsychiat.*, 1961, V. 2, 5125.

3. Weissbach, H., Smith, T. G., Daly, G. W., Witkop, B., Udenfriend, S., *J. Biol. Chem.*, 1960, V. 235, 1160.

4. Dixon, M., Webb, E. C., *Enzymes,* Academic Press, New York, 1958.

5. Toennies, G., Gallant, D. L., *Growth,* 1949, V. 13, 7.

Received Aug. 2, 1961. P.S.E.B.M., 1961, v108.