OBSERVATIONS ON THE MECHANISM OF ACTION OF TRANQUILLISERS—A STUDY OF THEIR EFFECT ON MONOAMINE OXIDASE, D- AND L-AMINO ACID OXIDASES AND CATALASE

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The effect of eight tranquillisers, widely differing chemically, on monoamine and L-amino acid oxidases of liver, D-amino acid oxidase of kidney and the catalase of liver, have been investigated. Reserpine was found to stimulate D- and L-amino acid oxidases and to inhibit monoamine oxidase. Rescinnamine was much less effective in this respect. Phenothiazine derivatives showed significant inhibitory action on the catalase activity. Benactyzine and diphenhydramine had a mild inhibitory action on monoamine oxidase while meprobamate and mephenesin did not show any effect.

THE fascinating problem of the mode of action of tranquillisers has been made more complicated by the diverse chemical nature of these agents. As the nature of biochemical lesions in mental disturbances is still obscure, the mechanism of the beneficial action of this group of drugs is not yet understood. Brodie, Shore and Pletscher[1] observed similarity between the central effects of 5-hydroxytryptamine (5-HT) and reserpine, both containing an indole unit. The central effects of reserpine may be mediated through the continuous release in the brain of 5-HT[2] which is then largely metabolised by monoamine oxidase[3]. The action of chlorpromazine has been associated with 5-HT antagonism[4], amongst other mechanisms, while the effect of meprobamate[5] in anxiety states may be due to selective block of interneuronal circuits and that of benactyzine may be related to anti-acetylcholine activity[6].

In this investigation, the possible interference of these drugs with some of the enzyme systems concerned with the metabolism of amines and amino acids has been studied. The effect of the representative members of the rauwolfia alkaloids, phenothiazine derivatives, alkyl diols and diphenylmethane derivatives on monoamine and amino acid oxidases and catalase activity, has been investigated.

EXPERIMENTAL

Method and Material

Reserpine, rescinnamine, chlorpromazine, promethazine, meprobamate, mephenesin, benactyzine and diphenhydramine were selected for the present study.

The solutions were made in distilled water (2 mg./ml.). With reserpine and rescinnamine, the solutions were prepared in a concentration of 0·25 mg./ml. in 0·1N H_2SO_4 by gentle warming and adjusting the final pH to 5·5. Once dissolved, the solutions remained stable at room temperature.

Estimation of Amine and Amino Acid Oxidases

The activities were measured in a Warburg apparatus using fresh liver and kidney tissues of the rat as enzyme preparations. The tissues were homogenised in a Waring micro blender and the final concentration adjusted to 100 mg. liver tissue/ml. in phosphate buffer, pH 7·2 for monoamine oxidase; 100 mg. kidney tissue/ml. in distilled water for D-amino acid oxidase and 300 mg. liver tissue/ml. in sodium phosphate buffer, pH 8·3 for L-amino acid oxidase. In the side bulb of the Warburg flask, 0·2 ml. of tyramine or 5-HT creatinine sulphate (0·25M) for monoamine oxidase, 0·4 ml. of DL-alanine (5 per cent), for D-amino acid oxidase and 0·2 ml. L-leucine (0·1M) for L-amino acid oxidase were kept as substrates and tipped in after setting the assembled manometers at 37°. The main space of the flask contained the enzyme preparation, buffers and graded doses of drugs. The centre well contained 0·2 ml. of 2N NaOH for absorbing CO_2. The rate of oxygen consumption was measured for 1 hour in the monoamine oxidase and D-amino acid oxidase and for 3 hours in the L-amino acid oxidase estimations.

Catalase Activity

This was estimated by the method of Euler and Josephson[7], using 0·1N H_2O_2 as substrate. Diluted rat liver homogenate in water was used as enzyme preparation. The unreacted hydrogen peroxide was titrated by the iodine thiosulphate method.

RESULTS

The effect of reserpine and rescinnamine on the enzyme systems is shown in Tables I, II and III. The figures are the mean of 8 experiments in each case.

It may be observed from Table I that reserpine and rescinnamine, 2·5 μg./ml., inhibit monoamine oxidase, while at 100 μg./ml., reserpine produces a 70 per cent inhibition which is 2·5 to 3 times more than that produced by rescinnamine in the same concentration.

TABLE I

EFFECTS OF RESERPINE AND RESCINNAMINE ON MONOAMINE OXIDASE OF RAT LIVER

Concentration μg./ml.	Reserpine		Rescinnamine	
	μl. O_2/hr.	Inhibition per cent ± S.D.	μl. O_2/hr.	Inhibition per cent ± S.D.
Control	39·00	—	51·00	—
2·5	37·83	3·0 ± 0·32	49·92	2·1 ± 0·20
5·0	33·40	14·3 ± 0·70	46·81	8·2 ± 0·70
20·0	21·76	44·2 ± 2·60	43·91	13·9 ± 0·60
80·0	15·32	60·7 ± 4·10	37·74	26·0 ± 1·70
100·0	11·66	70·1 ± 3·50	37·75	26·0 ± 1·50

Chlorpromazine and promethazine were also found to inhibit monoamine oxidase but to a lesser extent than reserpine. In a lower concentration, 4 to 8 μg./ml., these drugs did not show any appreciable action and only 45 per cent depression was observed at 160 μg./ml.

Benactyzine and diphenhydramine were required in even higher doses for depressing the enzymatic activity. Diphenhydramine, at 320 μg./ml., could elicit only a 45 per cent depression. The relative potency of diphenhydramine to benactyzine was found to be 3 : 1. Meprobamate and mephenesin did not show any inhibitory action on monoamine oxidase.

TABLE II

EFFECTS OF RESERPINE AND RESCINNAMINE ON D-AMINO ACID OXIDASE OF RAT KIDNEY

Concentration μg/ml.	Reserpine		Rescinnamine	
	μl. O_2/hr.	Stimulation per cent ± S.D.	μl. O_2/hr.	Stimulation per cent ± S.D.
Control	75·00	—	69·00	—
2·0	80·25	7·0 ± 0·80	69·00	—
4·0	87·08	16·1 ± 0·90	69·80	1·1 ± 0·16
20·0	94·73	26·3 ± 1·60	77·63	12·5 ± 0·80
80·0	120·75	61·0 ± 3·50	90·05	30·5 ± 1·70
160·0	117·90	57·2 ± 4·20	86·60	25·5 ± 1·90

From Table II, it will be observed that reserpine, 2 μg./ml., stimulated D-amino acid oxidase, this reached 60 per cent with 80 μg./ml. Rescinnamine produced only 30 per cent stimulation at the same concentration.

Chlorpromazine, promethazine, diphenhydramine, benactyzine, meprobamate and mephenesin did not show any appreciable action on D-amino acid oxidase even at 400 μg./ml.

TABLE III

EFFECTS OF RESERPINE ON L-AMINO ACID OXIDASE OF RAT LIVER

Concentration μg./ml.	Period of incubation					
	1st hour		2nd hour		3rd hour	
	μl. O_2	Stimulation per cent ± S.D.	μl. O_2	Stimulation per cent ± S.D.	μl. O_2	Stimulation per cent ± S.D.
Control	5·0	—	9·0	—	11·0	—
5·0	6·6	32·5 ± 2·0	13·5	50·0 ± 2·6	18·0	63·3 ± 4·6
10·0	9·0	80·0 ± 3·8	22·5	150·0 ± 8·4	29·0	163·6 ± 9·2
25·0	13·0	160·0 ± 9·4	28·0	211·1 ± 10·2	36·0	227·2 ± 13·6
50·0	13·5	170·0 ± 8·6	29·1	223·3 ± 9·6	34·0	209·0 ± 12·1

From analysis of Table III, it will be seen that reserpine showed a marked stimulatory action on L-amino acid oxidase. At 5 μg./ml. 32, 50 and 63 per cent stimulation was observed during the first, second and third hour of incubation respectively. The stimulation reached 200 per cent at 25 and 50 μg./ml. With rescinnamine at the higher concentration, the stimulation did not exceed 27 per cent.

Inhibition of catalase activity was observed with chlorpromazine and promethazine where 10 μg./ml. produced a 10 to 14 per cent inhibition which was increased to 42 per cent at 100 μg./ml. The other drugs did not show any effect on L-amino acid oxidase and catalase enzyme systems.

DISCUSSION

Reserpine is known to deplete noradrenaline and 5-HT contents of brain[8]. Our observations indicate that reserpine can affect different

enzymes concerned with amino acid and amine metabolism. The diminished 5-HT level may be due to the reduced rate of synthesis but there is no evidence that the amount of metabolites of 5-HT in urine is reduced by reserpine. Stimulation of L-amino acid oxidase by reserpine might be responsible for the low level of 5-HT because of a quicker oxidation of tryptophan[9]. Rescinnamine, which is pharmacologically weaker than reserpine, shows less action on these enzyme systems.

Brain 5-HT levels are not affected by chlorpromazine[10]. This might be due to the drug's lack of action on amino acid oxidase.

The phenothiazine derivatives inhibit catalase activity, an observation which seems to be significant. The hydrogen peroxide formed during the oxidation of amino acids and amines is disposed of rapidly by the catalase enzyme system and the possibility of a narcobiotic action of chlorpromazine, due to accumulation of H_2O_2, may deserve consideration.

According to Berger[10], benactyzine does not increase the excretion of 5-hydroxyindole acetic acid in urine. In our investigation also it did not have any effect on amino acid oxidase.

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