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INHIBITION OF AMINE OXIDASE BY ANTIHISTAMINE COMPOUNDS AND RELATED DRUGS

BY

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Despite the considerable amount of work on the pharmacology of antihistamine drugs since their introduction by Bovet and Staub (1937), relatively little appears to be known concerning the actions of these substances on enzymes. From a consideration of the pharmacological properties of the antihistamines, the enzymes which would be most likely to be affected are histaminase and amine oxidase.

Kapeller-Adler (1949) studied the effect of six antihistamine drugs on a purified hog kidney histaminase preparation, and found that four of them caused no change in enzymic activity, although two did partially inhibit this enzyme. In the course of earlier unpublished experiments on amine oxidase Thompson and Tickner observed that diphenhydramine hydrochloride inhibits this enzyme. Schuler (1950) has also shown that antistin inhibits amine oxidase. Search of the literature showed that potentiation of adrenaline was a common feature of antihistamine drugs. A more systematic study of this inhibitory effect was therefore made. It is the purpose of this paper to show that all antihistamine drugs so far tested are inhibitors of amine oxidase.

Materials and Methods

The following inhibitors were used:

1. Diphenhydramine hydrochloride (Benadryl, Parke Davis & Co.).
2. Tripelennamine hydrochloride (Pyribenzamine, Ciba Laboratory).
3. Antazoline sulphate (Antistin sulphate, Ciba Laboratory).
4. Mepyramine hydrogen maleate (Anthisan, May & Baker Ltd.).
5. Promethazine hydrochloride (Phenergan, May & Baker Ltd.).
6. Phenindamine hydrogen tartrate (Thephorin tartrate, Roche Products Ltd.).
7. Piperidinomethyl-benzodioxane (933F).
8. N : N-dibenzyl-β-chloroethylamine (Dibenamine hydrochloride, Smith, Kline & French).

The source of the amine oxidase preparation was fresh rat liver. In two experiments guinea-pig kidney and rabbit artery were used.

Tyramine hydrochloride (L. Light & Co.) in 0.01 M solution was used as substrate throughout except in those experiments in which the relation between substrate concentration and oxidation ratio was studied.

The method employed was as follows. Albino rats of either sex were stunned and decapitated. The liver was removed, weighed, and homogenized in 0.067 M-phosphate

buffer (*p*H 7.3) so that each ml. of homogenate contained 100 mg. fresh wt. of liver; 2 ml. homogenate (=200 mg. of liver) were placed in the main chamber of a Warburg flask. The inhibitors were dissolved in glass-distilled water and brought to *p*H 7.3 with N-HCl or N-NaOH as required. The final concentrations of inhibitor varied from 2×10^{-5} to 2×10^{-2} M, and the actual volumes of solution added to the main chamber varied between 0.1 and 0.5 ml.; 0.2 ml. of 0.01 M-tyramine hydrochloride was placed in the side bulb. The period of time during which the drug remained in contact with the enzyme preparation before the addition of substrates averaged 15 min. 0.2 ml. KCN solution was added to the main chamber to give a final concentration of 0.001 M; in the centre well was placed 0.2 ml. of a mixture of equal volumes of 2 M-KCN and 0.002 M-KOH.

The level of activity of the uninhibited enzyme was determined in each experiment, controls being included for cyanide-insensitive residual respiration. Where the inhibiting compound was in the form of a salt, the sodium salt of the anion was also added to the control flasks in the same molarity.

The flasks were gassed with O_2, equilibrated at 38° C. for 10 min., the taps closed, and the levels noted. Tyramine was tipped into the main chamber and 30 min. later the levels again read.

When guinea-pig kidney was used as the enzyme source, the experiment was continued for 60 min., and with rabbit arteries 120 min.

In determining the type of inhibition the substrate concentration varied from 10^{-3} to 10^{-2} M.

RESULTS

The results are shown in Table I. It will be seen that all the substances investigated inhibit the enzyme, the degree of inhibition being proportional to the log concentration of the drug. In the higher concentrations there was usually little

TABLE I

Inhibitor	Molar concentration		
	10^{-4}	10^{-3}	10^{-2}
	% inhibition		
Diphenhydramine	22	49	79
Tripelennamine	18	44	82
Antazoline	9	36	77
Mepyramine	9	32	81
Promethazine	25	48	93
Phenindamine	10	45	—
933F	0	22	74
Dibenamine	0	12	—

difference between the drugs, but in the region of 10^{-4} M some quantitative differences did emerge, the most striking of which was the lack of inhibition by 933F and by dibenamine.

Dibenamine, however, because of its very low solubility in aqueous solutions at *p*H 7.3, could be studied only over a very limited range of concentrations, and results with it cannot be as reliable as with the other drugs.

Fig. 1 shows the results obtained for diphenhydramine with three different tissues from three different species. The purpose of these experiments was to show that inhibition of this enzyme is not restricted to one species or one tissue.

The type of inhibition exerted by the antihistamines studied was investigated by plotting the reciprocals of the reaction velocity at various substrate concentrations in presence and absence of inhibitor (Lineweaver and Burke, 1934). Parallel lines were obtained (Fig. 2) showing that the inhibition was non-competitive.

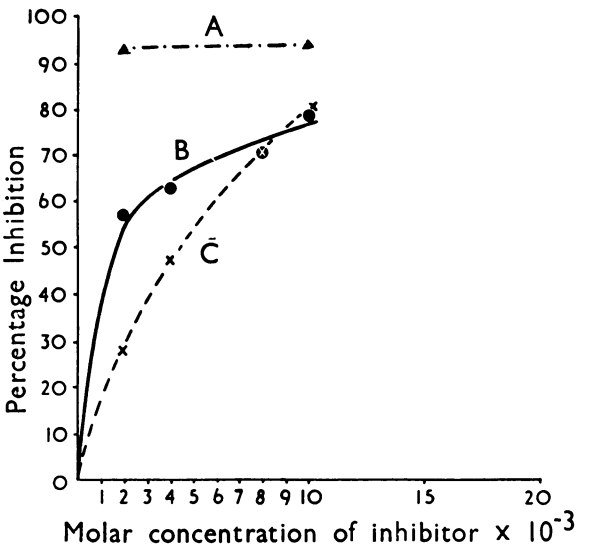

FIG. 1.—Inhibition of amine oxidase from different sources by diphenhydramine. Curve A: minced rabbit aorta; curve B: rat liver homogenate; curve C: guinea-pig kidney homogenate.

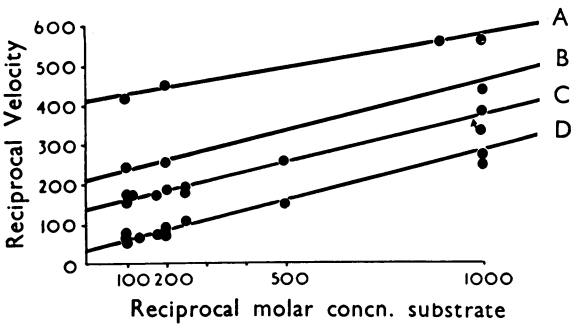

FIG. 2.—Non-competitive nature of inhibition of amine oxidase (rat liver) by diphenhydramine. Ordinates: reciprocal velocities (μl.O_2/g. tissue/hr.). Abscissae: reciprocal molar concentrations of substrate. Concentrations of diphenhydramine hydrochloride: A, 5×10^{-3}M; B, 3×10^{-3}M; C, 10^{-3}M; D, none.

DISCUSSION

The numerous substances which inhibit amine oxidase include sympathomimetic drugs such as amphetamine (Pugh and Quastel, 1937) and ephedrine (Blaschko, Richter, and Schlossman, 1937), local anaesthetics such as cocaine, cinchocaine (Philpot, 1940), and amethocaine (Tickner, unpublished results), and sympathomimetic and sympatholytic imidazolines (Meier, 1950).

Many of these substances imitate or potentiate adrenaline and some antagonize its actions ; these effects have been explained as being due to inhibition of amine oxidase or to combination with adrenaline receptors respectively.

Antihistamine substances have been shown to potentiate adrenaline (e.g., De Cuyper, 1946 ; Parrot, 1943 ; Loew, MacMillan, and Kaiser, 1946 ; Sherrod, Loew, and Schloemer, 1947 ; Sherrod, Schloemer, and Loew, 1946 ; Yonkman, Chess, Mathieson, and Hansen, 1946), or to produce a rise in blood pressure (e.g., Winder and Thomas, 1947 ; Orias, Gilbert, and Brooks, 1949 ; Haley and Harris, 1949 ; Allardyce, Salter, and Rixon, 1951).

The present observations made *in vitro* may explain these side-effects, particularly as the inhibition is non-competitive, unlike that due to ephedrine and the local anaesthetics.

As Tripod (1940) has pointed out the quantitative relationship between the anaesthetic power of local anaesthetics and their sympathomimetic action is not known. Nevertheless it is of interest that the antihistamine drugs studied are both inhibitors of amine oxidase and also local anaesthetics (Brach, 1946 ; Climento, Homburger, and Mosser, 1941 ; Dews and Graham, 1946 ; Halpern, 1942 ; Loew, Kaiser, and Moore, 1945 ; Rosenthal and Minard, 1939 ; Lehmann, 1948).

Another property of these drugs shared with the local anaesthetics is that of having a quinidine-like action on the heart (Dews and Graham, 1946 ; Dutta, 1949). For this reason the effects of quinidine and of atropine were tested on amine oxidase. No inhibition was observed either at 10^{-3} or 10^{-4} M under the conditions described above.

Finally the antihistamines have been used by a number of workers in pharmacological experiments, involving adrenaline, in order to eliminate the effects of histamine. Their sympathomimetic and sympatholytic side-actions indicate that conclusions from such experiments should be drawn with caution.

SUMMARY

1. Six antihistamine drugs and two anti-adrenaline drugs inhibit amine oxidase.

2. The possible relationship between this finding and the pharmacology of these drugs is discussed.

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