appropriate regions of the chromatograms. Provided that none of these compounds was present in excess, their presence did not interfere with the assay of NA. The enzyme procedure can in principle be applied to the assay of any of these alternative substrates; a method for the assay of octopamine in this way has been described.[2] We have also applied the present method to octopamine assays with satisfactory results (Table 2). The assay of NA in freshly prepared tissue homogenates is simpler and more reliable than the use of perchloric acid extracts.[1] In our hands, it has proved unsatisfactory to use perchloric acid extracts because of the difficulty of neutralizing and removing the perchloric acid prior to assay. The use of magnesium carbonate for this purpose, as advocated by Saelens *et al.*,[1] tended to produce samples with an alkaline pH in which the catecholamines were extremely unstable. Potassium phosphate or TRIS were not suitable as neutralizing agents because these substances when present in high concentrations interfered with the enzyme reaction. The presence of catecholamine metabolizing enzymes in freshly prepared tissue homogenates does not interfere with the assay since the reaction mixture contains inhibitors of both monoamine oxidase and catechol-*O*-methyltransferase.

The method described is suitable for the assay of NA in small aliquots of homogenates of tissues which contain relatively high concentrations of endogenous NA or in aliquots of samples from sucrose density gradients.[3, 4] For such samples this method has the advantage of allowing NA to be assayed on a small aliquot, leaving material which can be used for the parallel assay of other components (enzymes etc.). In other tissues, such as brain, however, in which the reaction did not consistently proceed to completion the method yielded unreliable results.

By reducing the volumes of incubation mixture and sample, it has proved possible to increase the sensitivity of the radiochemical assay procedure to less than 0·1 ng for L-NA, so that for the micro-assay of NA in very small samples of tissue the enzyme assay has obvious advantages and possibilities for future use. Preliminary results suggest that it may be possible to control to some extent for the inconsistent completion of the reaction in certain tissues by incorporating a double isotope modification, in which $^{14}$C-NA is added to the tissue samples before assay, and the ratio of $^{3}$H/$^{14}$C is measured in the product adrenaline.

*Acknowledgements*—This study is supported by a grant from the Mental Health Research Fund to L. L. Iversen. B. Jarrott gratefully acknowledges support from Kodak (Australasia) Ltd.

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Biochemical Pharmacology, Vol. 19, pp. 1843–1845. Pergamon Press. 1970. Printed in Great Britain

## The effect of antihistamines on red blood cell acetylcholinesterase activity *in vitro*

(*Received* 17 *October* 1969; *accepted* 2 *December* 1969)

Several reports concerning the pseudocholinesterase-inhibitor effect of certain antihistamines have appeared; Vincent[1] reported the inhibitory effect of Antergan, Todrick[2] that of promethazin. Benstz, observed the same effect of Phenergan and chlorpromazine, and also determined their influence on

the acetylcholinesterase (AChE; acetylcholine hydrolase; EC 3.1.1.7.) activity, but in the doses administered it was found unaltered.

The inhibitory effect of chlorpromazine on pseudocholinesterase and AChE activity is well known, both from *in vitro*[4–11] and *in vivo* experiments.[3, 12] Erdös *et al.* have demonstrated[9] that the effective derivative of chlorpromazine, chlorpromazine sulfoxide[13] proved to be less potent inhibitor, than chlorpromazine. Arterberry[14] administered chlorpromazine to a patient, poisoned with the cholinesterase inhibitor phosdrin, with fatal effect.

*Materials and methods*

The following materials were used: chloropyramine (Suprastin, Richter), tripelennamine (Richter), promethazine HCl (E.G.Y.T., Budapest), phenindamine bitartrate (Chinoin), chlorpromazine (Hibernal, E.G.Y.T.), Thenalidine (Sandosten-Calcium, Sandoz), acetyl-β-methyl choline chloride (MeCh; Th. Schuchardt, Munich) in 25 mM/l. concentration dissolved in M/15 acetate buffer (pH 4·5), histamine hydrochloride (Peremin, Chinoin), M/15 pH 7·8 barbital buffer.

The blood samples of young male rats were centrifuged, washed by Ringer solution, hemolysed by water and the hemoglobin removed by washing. The stroma of 0·25 cc blood was suspended in 1·0 cc distilled water and was added to this system 0·4 cc MeCh and 0·6 cc of the material to be tested. The volume of each tube was completed to a volume of 4·0 cc by barbital buffer. The mixture was incubated at 37° for 30 min (in the cases of kinetic analysis for 20 min). The enzyme reaction was stopped by the addition of trichloroacetic acid and the MeCh content of the solution determined by the method of Hestrin.[15] The kinetic analysis was carried out according to the method of Lineweaver and Burk.[16]

*Results and discussion*

The rate of inhibition has been expressed in the percentage of the actual normal activity. The normal value means the enzyme activity determined simultaneously with the antihistamine containing tubes from the same blood dilution. In our system the normal activity generally was 1·0–1·6 mM/l./hr MeCh split. Antihistamines in a concentration of about $10^{-4}$ M/l. significantly inhibited the AChE activity, except thenalidine which up to a concentration of $2 \times 10^{-3}$ M/l. was without effect (see Fig. 1).

The kinetic evaluations in all cases have demonstrated a competitive type of inhibition.

Histamine in a concentration of $10^{-4}$ M/l. could not prevent the effect of antihistamines.

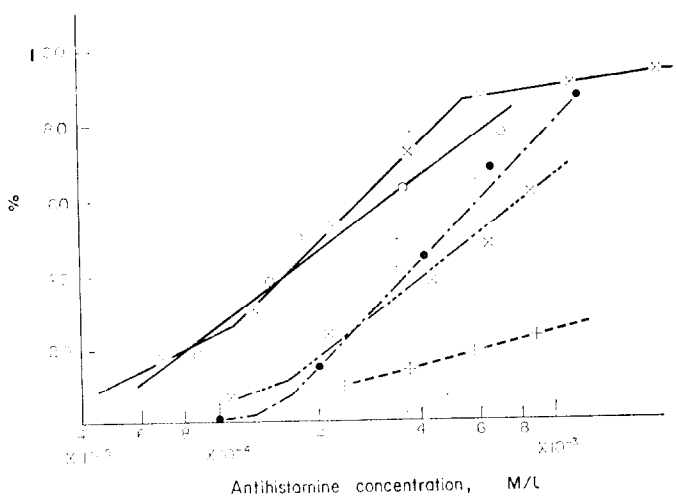
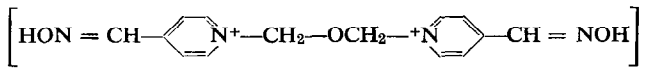



FIG. 1. Inhibition of red blood cell acetylcholinesterase activity (expressed in the percentage of the control) plotted against the final concentration (M/l.) of the antihistamines (chloropyramine: ×—×, chlorpromazine: ○—○, phenindamine tartrate: × - - - ×, promethazine HCl: ×—· · ·—×, tripelennamine HCl: (- · · · -) administered.

Washing twice by distilled water (10·0 cc in water in each time) did not result in any change in the inhibition. Toxogonin ($10^{-3}$ M/l.) added prior to the system did not influence the inhibitory effect of antihistamines.



Although in the present experiments we could demonstrate an *in vitro* AChE inhibiting effect of several antihistamines, the same inhibitory effect could hardly be supposed *in vivo*. In our recent preliminary experiments no remarkable inhibition of the red blood cell AChE activity could be proved after *in vivo* administration of chloropyramine. No correlation has been found between the histamine-antagonising and AChE-inhibiting doses of antihistamines. Histamine itself has also failed to prevent the AChE inhibition. However, some of the side effects of antihistamines might have a connection with the inhibition of cholinesterases. Considering the fact that antihistamines are unevenly distributed within the organism, one can suppose that the administration of antihistamines potentiates the *in vivo* effects of anticholinesterases, as in the case of Arterberry chlorpromazine did it.

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