*Brit. J. Pharmacol.* (1957), **12**, 320.

# THE ENZYMATIC HYDROLYSIS OF IMIDAZOLEACRYLOYLCHOLINE (MUREXINE) AND IMIDAZOLEPROPIONYLCHOLINE (DIHYDROMUREXINE) BY VARIOUS CHOLINESTERASES

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(RECEIVED MARCH 2, 1957)

Several choline esters, including the imidazoleacryloyl and imidazolepropionyl compounds, have been hydrolysed by cholinesterases from various sources. The imidazolepropionyl ester was metabolized by cholinesterases obtained from human plasma, ox spleen, ox serum, and guinea-pig liver, but not by rat liver or bovine red cell cholinesterase. It is suggested the imidazolepropionylcholine or a closely related ester might be the natural substrate for "non-specific" cholinesterase.

Vincent and Jullien (1938) reported that extracts of the hypobranchial gland of *Murex* caused contraction of the leech muscle. This action was abolished by incubating these extracts in serum, an indication that the substance which caused the contraction was metabolized. Later, Erspamer and Dordoni (1947) described the presence of a choline ester in the hypobranchial gland of the snail, *Murex trunculus*. This ester was identified (Erspamer and Benati, 1953) as (2-$\beta$-imidazol-4(5)-ylacryloyloxyethyl)trimethylammonium chloride hydrochloride variously named murexine, urocanylcholine, or imidazoleacryloylcholine (ImAcrCh). ImAcrCh and its analogue, imidazolepropionylcholine (ImPrCh), stimulate autonomic ganglia, interfere with transmission at the neuromuscular junction, and potentiate the effects of histamine on smooth muscle (Erspamer and Dordoni, 1947; Kewitz, 1955; Conroy, Kappell, Ferruggia, and Randall, 1956; Tabachnick and Roth, 1957; Winbury, Wolf, and Tabachnick, 1957).

Since Grunert and Kewitz (1955) have demonstrated that an imidazoleacylcholine ester occurs naturally in horse, rat, and bovine brain, the study of the hydrolysis of imidazoleacylcholine esters assumes some significance. Therefore, it was of interest to ascertain whether ImAcrCh and its analogue, ImPrCh, would serve as substrates for several cholinesterases.

## MATERIALS AND METHODS

The esterase determinations were performed by a modification of the method of Ammon (1933) at 37° with a gas phase of 95% nitrogen and 5% carbon dioxide. Sufficient $NaHCO_3$ was used to give a final concentration of 0.07M, *p*H 8.0.

Enzyme, buffer and water were placed in the main compartment and substrate and inhibitor in the sidearms. After gassing and temperature equilibration, the substrate was added. If an inhibitor was present, it was added from the sidearm simultaneously with the substrate. In most experiments, readings were taken at 3 to 6 min. intervals.

Various cholinesterases were used. Acetylcholinesterase was prepared from bovine red blood cells (Winthrop-Stearn). Cholinesterase of human plasma (Cholase$^R$) was obtained as a concentrate, 1 ml. of the preparation representing the cholinesterase content of 350 ml. of original plasma. Rat and guinea-pig liver esterases were prepared by homogenizing 1 part minced liver in 4 parts of cold 0.85% NaCl in a Potter–Elvejhem homogenizer. Bovine spleen esterase was obtained by removing the outer sheath and fat from a portion of spleen and preparing the underlying tissue as described for rat and guinea-pig liver. Bovine serum and spleen were obtained from freshly killed steers.

The following substrates were used: Imidazoleacryloylcholine chloride hydrochloride; imidazolepropionylcholine bromide (Hoffmann-LaRoche); butyrylcholine chloride (J. H. DeLamar and Son); propionylcholine iodide (Dajac Laboratories);

benzoylcholine chloride (J. H. DeLamar and Son); triacetin (Eastman); and methacholine (Sharpe and Dohme).

## RESULTS

The velocities of hydrolysis of certain choline esters in final concentrations of $10^{-2}$M to $10^{-3.5}$M are given in Table I. ImPrCh and butyrylcholine (BuCh) were hydrolysed at approximately equal rates by human plasma non-specific cholinesterase. The respective Km values for these esters calculated from initial velocities were $1.37 \times 10^{-3}$M and $1.53 \times 10^{-3}$M. Other substrates tested in this system in decreasing order of activity were propionylcholine (PrCh), benzoylcholine (BzCh), and ImAcrCh. Methacholine (MeCh) was hydrolysed by specific cholinesterase while ImAcrCh and ImPrCh were not metabolized by this enzyme. The inhibition of the hydrolysis of ImPrCh and BuCh by $10^{-5}$M eserine with a preparation of human cholinesterase was 87 and 81% respectively.

TABLE I

THE VELOCITIES OF THE ENZYMATIC HYDROLYSIS OF SOME CHOLINE ESTERS BY BOVINE RED BLOOD CELL (SPECIFIC) CHOLINESTERASE (15 UNITS/FLASK) OR BY HUMAN PLASMA (NON-SPECIFIC) CHOLINESTERASE (0.2 ML. OF A 1:375 DILUTION OF CHOLASE/FLASK)

Final volume 2 ml.

| Substrate | Cholinesterase | Initial Velocities Expressed as $\mu$l.$CO_2$ Evolved/1 hr. | | | |
|---|---|---|---|---|---|
| | | $10^{-2}$M | $10^{-2.5}$M | $10^{-3}$M | $10^{-3.5}$M |
| Methacholine .. .. | Specific | 235 | 186 | 118 | 36 |
| Imidazolepropionylcholine | ,, | — | 0 | 0 | 0 |
| Imidazoleacryloylcholine | ,, | 0 | 0 | 0 | 0 |
| Imidazolepropionylcholine | Non-specific | 980 | 735 | 305 | 150 |
| Imidazoleacryloylcholine | ,, | 40 | 55 | 61 | 37 |
| Benzoylcholine .. .. | ,, | 280 | 295 | 140 | 48 |
| Propionylcholine .. .. | ,, | 737 | 566 | 300 | 54 |
| Butyrylcholine .. .. | ,, | 990 | 775 | 375 | — |

The inhibition of the hydrolysis of ImPrCh and BuCh by $10^{-5}$M eserine with a preparation of human cholinesterase was 87 and 81% respectively.

Various tissues were examined for their cholinesterase activity (Table II). Guinea-pig liver hydrolysed both BzCh and ImPrCh. Rat liver did not hydrolyse ImPrCh but metabolized triacetin at a very high rate. Some activity was observed with BuCh. Ox serum- and spleen-cholinesterases hydrolysed ImPrCh and BuCh, but not BzCh nor ImAcrCh. The rate of hydrolysis of ImPrCh was somewhat greater than that of BuCh.

TABLE II

THE ENZYMATIC HYDROLYSIS OF CERTAIN CHOLINE ESTERS BY VARIOUS TISSUES

Enzyme/flask: 1 ml. of 20% homogenate of ox spleen, rat or guinea-pig liver, or 1 ml. of ox serum. Final volume 2 ml. (*a*) denotes that the substrate concentration was $10^{-2.5}$M, (*b*) that 15 min. readings and (*c*) that 3 min. readings were extrapolated to obtain the 30 min. values.

| Substrate ($10^{-2}$M) | Eserine ($10^{-5}$M) | $\mu$l. $CO_2$ Evolved/30 Min./Flask | | | |
|---|---|---|---|---|---|
| | | Ox Spleen | Ox Serum | Rat Liver | G. Pig Liver (*b*) |
| Imidazoleacryloylcholine | 0 | 0 | 0 | | |
| Imidazolepropionylcholine | 0 | 35 | 36 | 0 | 152 |
| ,, | + | | | | 0 |
| Butyrylcholine .. .. | 0 | 25 | 27 | 45 | |
| Benzoylcholine .. .. | 0 | 0 | 0 | | 176 (*a*) |
| ,, .. .. | + | | | | 164 (*a*) |
| Methacholine .. .. | 0 | 24 | | | |
| Triacetin .. .. .. | 0 | | | 1,960(*c*) | |

A concentration of $10^{-5}$M eserine, which inhibited the hydrolysis of BzCh by guinea-pig liver esterase only slightly, completely inhibited the hydrolysis of ImPrCh (Table II).

## DISCUSSION

The work reported here suggests some specificity of the "non-specific" cholinesterase for ImPrCh. The evidence for this is as follows: (*a*) The non-specific cholinesterase of human serum hydrolyses ImPrCh more rapidly than ImAcrCh, PrCh, and BzCh. However, BuCh is metabolized slightly more rapidly than ImPrCh. (*b*) ImPrCh and ImAcrCh are not metabolized by the specific esterase of bovine red blood cells, whereas BuCh and BzCh are metabolized by specific cholinesterase though at a slow rate (Adams, 1949). (*c*) The non-specific cholinesterase of ruminant serum and spleen which does not act on BzCh (Gunter, 1946), but which does metabolize BuCh (Bannister, Whittaker, and Wijesundera, 1953; Mendel and Myers, 1955; Hardwick, 1956), has now been shown to hydrolyse ImPrCh more rapidly than BuCh. (*d*) Rat liver homogenates were shown to metabolize triacetin rapidly, BuCh very slowly, and ImPrCh not at all. (*e*) Sawyer (1945) reported the presence of a benzoylcholinesterase in guinea-pig liver which was not inhibited by eserine. We have confirmed this, but have observed also that this tissue rapidly metabolized ImPrCh. The hydrolysis of ImPrCh was prevented by eserine ($10^{-5}$M final concentration), and therefore the enzyme which metabolized ImPrCh cannot be benzoylcholinesterase.

It would appear that ImPrCh might be as effective as BuCh as a substrate for non-specific cholinesterase. It is conceivable that the specificity pattern displayed by ImPrCh for some cholinesterases would also be displayed by an imidazoleacylcholine ester that occurs in the organism. If this were the case, one might infer a need for the so-called non-specific esterase, namely, for the metabolism of an imidazoleacylcholine ester.

Since this paper was written Foldes, Erdös, Baart, and Shanor (1957) have reported the hydrolysis of imidazoleacryloylcholine and imidazolepropionylcholine by human plasma and red cell cholinesterase.

The authors are grateful to Dr. L. Randall and Dr. V. Erspamer for their generous gifts of imidazolepropionylcholine bromide and imidazoleacryloylcholine chloride hydrochloride respectively, and to Dr. V. Seeberg, Cutter Laboratories, for his kindness in making the Cholase$^{R}$ available. The authors wish to express their appreciation to Miss J. Mershon for her assistance.

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