Biochemical Pharmacology, 1962, Vol. 11, pp. 487–491. Pergamon Press Ltd., Printed in Great Britain.

ENZYMIC HYDROLYSIS OF SATURATED AND UNSATURATED ACID ESTERS OF CHOLINE*

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(*Received* 3 *November* 1961; *accepted* 20 *February* 1962)

Abstract—Rates of hydrolysis of several saturated and unsaturated acid esters of choline by purified serum and erythrocyte cholinesterase have been examined. In general, the introduction of a double bond in the α, β-position markedly reduced the rate of hydrolysis by both enzymes, as compared with that of the saturated homolog. The results are discussed in the light of the current concepts of enzymic hydrolysis of cholinesters and the principles of physical organic chemistry.

SEKUL AND HOLLAND[1, 2] recently have examined the effect of several saturated and unsaturated acid esters of choline on the blood pressure of eserinized dogs and cats. It was observed that the introduction of a double bond in the α,β-position of the acyl group diminished or abolished the depressor (muscarine-like) and enhanced the pressor (nicotine-like) activity of the saturated homolog. It was postulated that the enhanced pressor activity resulted from an increase in the formal negative charge density on the carbonyl oxygen induced by resonance (+ R effect) between the carbonyl group and the α,β-double bond.

In conjunction with these studies, we have compared the rates of hydrolysis of a number of these esters with those of acetylcholine by purified bovine erythrocyte and human serum cholinesterase. The investigations are summarized and briefly discussed.

EXPERIMENTAL

Cholinesterase activity was measured manometrically by the standard Warburg technique. Each manometer vessel contained 200 μg of purified human serum cholinesterase or 2·5 μg of purified bovine erythrocyte cholinesterase. Substrates dissolved in 0·9% NaCl solution were added to the side bulb. Sufficient $NaHCO_3$ solution (0·154 M) was added to adjust the final volume to 2·0 ml. The manometers were gassed 15 min with 5% CO_2 — 95% N_2 mixture. All experiments were carried out at 37 °C. Purified serum cholinesterase (fraction IV-4 Run 457) was obtained from Protein Foundation Laboratories, Jamaica Plain, Mass., and the erythrocyte enzyme from Mann Research Laboratories, New York. The synthesis of and the analytical data concerning the esters employed can be found in a previous publication.[2] The data are presented as relative rates of hydrolysis, that of acetylcholine being taken as 1. Only the initial rates of hydrolysis (first 15 min) were used in these calculations. Values presented represent the mean of at least 15 observations. All data have been corrected for

* This research was supported in part by U.S. Public Health Service grants H-4265 and H-6072.

spontaneous rates of hydrolysis. In preliminary studies, human erythrocytes and serum were employed. The relative rates of hydrolysis of the various esters were not significantly different from those reported in the present communication. For this reason these data are not presented.

RESULTS

The results of these studies are summarized in Table 1. There are several features

TABLE 1. RELATIVE RATES OF HYDROLYSIS OF SEVERAL SATURATED AND UNSATURATED ACID ESTERS OF CHOLINE

Esters	Structure*	Relative cholinesterase activity Serum	RBC
Acetylcholine	CH_3COO-R	1·00	1·00
Propionylcholine	CH_3CH_2COO-R	1·86	0·62
Butyrylcholine	$CH_3(CH_2)_2COO$-R	2·39	0·02
Valerylcholine	$CH_3(CH_2)_3COO$-R	1·95	0·01
α-Et-Butyrylcholine	$CH_3CH_2CH(C_2H_5)COO$-R	0·16	0·00
α-Me-Butyrylcholine	$CH_3CH_2CH(CH_3)COO$-R	0·13	0·01
Acrylylcholine	$CH_2=CHCOO$-R	1·01	0·12
Crotonylcholine	$CH_3CH=CHCOO$-R	0·27	0·02
Vinylacetylcholine	$CH_2=CHCH_2COO$-R	1·86	0·11
2-Pentenoylcholine	$CH_3CH_2CH=CHCOO$-R	0·10	0·00
4-Pentenoylcholine	$CH_2=CH(CH_2)_2COO$-R	2·20	0·00
α-Me-Acrylylcholine	$CH_2=CH(CH_3)COO$-R	0·47	0·20
α-Me-Crotonylcholine	$CH_3CH=C(CH_3)COO$-R	0·11	0·00
β-Me-Crotonylcholine	$(CH_3)_2$-C=CH-COO-R	0·00	0·00
α-Et-Crotonylcholine	$CH_3CH=C(C_2H_5)COO$-R	0·09	0·00

Final concentration of substrates was 1×10^{-2} M with the human serum enzyme and 5×10^{-3} M with the bovine erythrocyte enzyme. Relative activities are expressed on a molar basis.

* R = $-CH_2-CH_2-\overset{+}{N}(CH_3)_3$

of the data that require comment. In general, the observed rates of hydrolysis of all esters examined were considerably lower with the bovine erythrocyte enzyme than with that of human serum. We have observed, as have others,[3] that an increase in the number of carbon atoms in the acyl group caused a progressive increase in the rate of hydrolysis of saturated esters by the serum enzyme and a progressive decline in rate with the red cell enzyme when compared with acetylcholine; in both cases, maximal effects were observed with acyl groups of 4 or 5 carbon atoms.

Introduction of a double bond in the α,β-position considerably reduced the rate of hydrolysis by both enzymes, when compared with the corresponding saturated homologs, α,β-unsaturation being more effective in case of the serum enzyme. In contradistinction to the saturated series, an increase in the number of carbon atoms in the acyl group of the unsaturated ester was accompanied by a progressive decline in hydrolysis rate by the serum enzyme (compare the relative rates of hydrolysis of acrylyl-, crotonyl-, and 2-pentenoyl-choline, respectively, with the corresponding saturated homologs). Introduction of a double bond in a position other than α,β- (e.g., vinylacetyl-, and 4-pentenoyl-choline) had no great effect on the hydrolysis rate. Substitution of alkyl groups in either the α- or the β-position in the acyl group of the

saturated and unsaturated esters further reduced the rate of hydrolysis by serum enzyme.

The effect of β-methyl crotonylcholine (senecioylcholine; SCh) on the rate of hydrolysis of acetylcholine by serum and erythrocyte cholinesterase was studied. This particular ester was chosen because of its negligible rate of hydrolysis. In Fig. 1 we

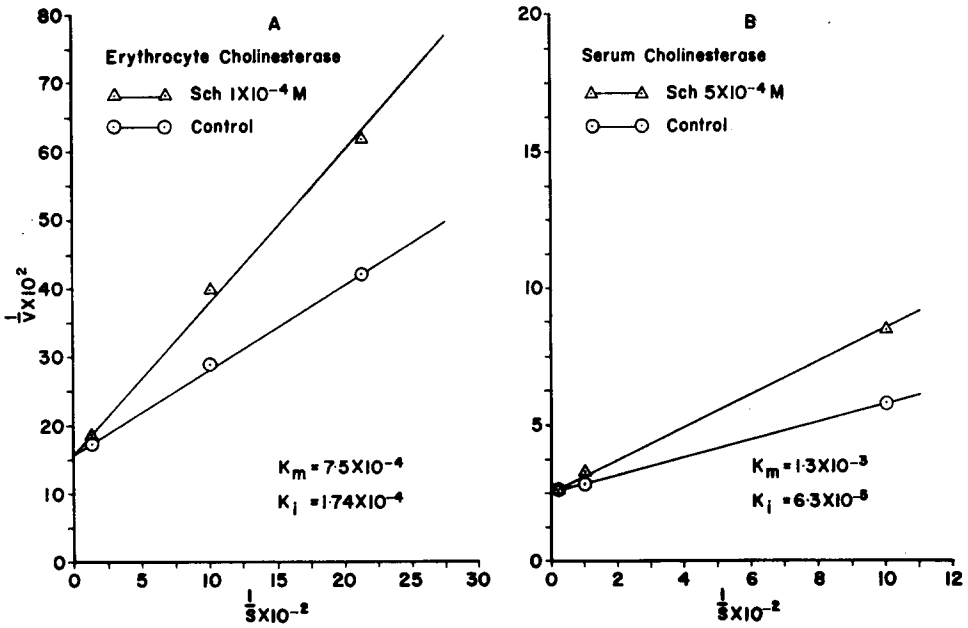


FIG. 1. Effect of β-methyl crotonylcholine on the enzymatic hydrolysis of acetylcholine by purified human serum and bovine erythrocyte cholinesterase.

present a Lineweaver and Burk[4] analysis of the data for the serum enzyme (Fig. 1B) and erythrocyte enzyme (Fig. 1A), using 5×10^{-4} M and 1×10^{-4} M of β-methyl crotonylcholine, respectively. β-Methyl crotonylcholine appeared to be a competitive inhibitor of both enzymes. The estimated dissociation constants are presented in the figure. From these it can be calculated that the affinities of the serum and red cell enzymes for β-methyl crotonylcholine are 20 and 5 times greater, respectively, than for acetylcholine.

DISCUSSION

The important points established in the present study can be summarized as follows. An increase in the number of carbon atoms in the acyl group of saturated choline esters was accompanied by a progressive decline in hydrolysis rate by erythrocyte cholinesterase and a progressive increase in rate by the serum enzyme. Introduction of a double bond in the α,β-position reduced the rate of hydrolysis by both enzymes. Introduction of a double bond in other positions was without consistent effect. Substitution of alkyl groups in the α-position in the saturated and unsaturated esters caused an additional reduction in the rate of hydrolysis.

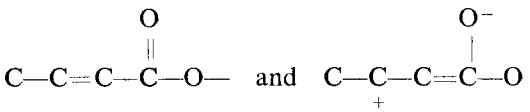
An interpretation of the findings can be facilitated by first considering briefly some of the proposed mechanisms of enzymatic hydrolysis of choline esters, as well as acid-base catalyzed hydrolysis of the corresponding ethyl esters.

The hydrolysis of acetylcholine by the red cell-type enzyme (acetylcholinesterase) was pictured by Wilson *et al.*[5] as involving interaction of the positively polarized carbonyl carbon and a nucleophilic group in the esteratic site. The subsequent formation of an acylated enzyme was thought to be an important intermediate step. In the hydrolysis of esters by serum cholinesterase, Ormerod[6] envisaged interaction of the negatively polarized carbonyl oxygen and a positively polarized (electrophilic) site on the enzyme. Both of these models emphasized the importance of electron activation in the acetoxy group during the initial steps of the hydrolytic process.

With the alkaline hydrolysis of the corresponding ethyl esters of a number of saturated and unsaturated acids, Davis and Evans[7] and Thomas and Watson [8] observed that either an increase in the length of the acyl group or the introduction of a double bond in the α,β-position was accompanied by a decrease in hydrolysis rate. They attributed their findings to an increase of the formal negative charge density on the carbonyl oxygen and a decreased positive charge density on the carbonyl carbon resulting from the operation of a fundamental electronic mechanism. The positive inductive effect (+I effect) was thought to be operative with the saturated esters and the resonance (+ R effect) or mesomeric effect (+ M effect) with the α,β-unsaturated esters. The electron-repelling effect of alkyl groups (+ I effect) decreases in the following order:

$$(CH_3)_3C > C_4H_9 > C_3H_7 > C_2H_5 > CH_3 > H$$

The resonance effect was pictured as operating in the following manner:



The altered charge densities in the carbonyl group were pictured as rendering the attack of the negatively charged hydroxyl ion progressively more difficult.

The observation that α,β-unsaturation reduced the rate of enzymic hydrolysis indicated that the resonance effect (+ R effect) was operative in this type also. Evidence to date (see above) suggests that an important step in enzymic hydrolysis of cholinesterase is the approach of a positively charged carbonyl carbon to a nucleophilic group in the esteratic site. The introduction of a double bond in the α,β-position would tend to reduce the positive charge on this carbon atom as the result of resonance. The decreased formal positive charge density may diminish binding by the esteratic site or the subsequent approach of the negatively charged hydroxyl ion, as proposed by Davis and Evans[7] and Thomas and Watson.[8] The observation that β-methyl crotonylcholine had a higher affinity for both enzymes than did acetylcholine and a negligible rate of hydrolysis would indicate that a hindered approach of the hydroxyl ion is, in all probability, responsible for the lower rates of hydrolysis of the α,β-unsaturated esters.

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