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Enzymatic Hydrolysis of Lactoyl- and Glyceroylcholines*

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Introduction

In connection with a study of the effect of the molecular constitution of synthetic entities upon isolated enzyme systems, we had the opportunity to observe the behaviour of new propionylcholine derivatives in isolated human plasma cholinesterase systems. Although additional experiments are contemplated with 'pseudo'- as well as acetylcholinesterase, we should like to report the hitherto obtained results.

The series of hydroxy substituted propionylcholines was designed to permit the evaluation of the effect which gradual changes in the chemical structure, or physical properties, or both might have upon biological response. The synthesis of the D(−)-, L(+)- and DL-lactoylcholines was reported earlier.[1] DL-lactoyl-DL-(β-methylcholine) and DL-glyceroylcholine† were prepared according to the same procedure; the experimental details will be reported in a forthcoming communication.[2]

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† DL-lactoyl-DL-(β-methylcholine) iodide, m.p. 122·8–124·4° (corr.). *Anal.* Calcd. for $C_9H_{20}INO_3$: C, 34·07; H, 6·35; I, 40·00; N, 4·42 per cent. Found: C, 34·09; H, 6·35; I, 39·80; N, 4·53 per cent.

DL-glyceroylcholine iodide, m.p. 104·0–105·5° (uncorr.). The last step of this synthesis was carried out in 1 : 1 anhydrous ethyl ether-chloroform, because of the insolubility of the intermediate 2-dimethylaminoethyl glycerate in ethyl ether. *Anal.* Calcd. for $C_8H_{18}INO_4$: C, 30·10; H, 5·68; I, 39·77; N, 4·39 per cent. Found: C, 29·86; H, 5·66; I, 39·3; N, 4·48 per cent. (Analyses by Drs. G. Weiler and F. B. Strauss, Oxford, England.)

Methods and Results

Cutter's Cholase (purified human plasma 'pseudo'-cholinesterase) was chosen for the initial experiments; the compounds were exposed to the enzyme in concentrations of approximately $8 \cdot 22 \times 10^{-3}$M along with propionylcholine iodide* and acetylcholine iodide† as reference reagents. The work was carried out on a GME–Lardy W–3 Warburg instrument, using 15 ml flasks. The Krebs–Ringer bicarbonate buffer, consisting of $2 \cdot 3 \times 10^{-2}$M $NaHCO_3$, $7 \cdot 5 \times 10^{-2}$M KCl, $7 \cdot 5 \times 10^{-2}$M NaCl and 4×10^{-2}M $MgCl_2 . 6\ H_2O$, was prepared according to Cohen (see Reference 3a); the final reaction mixture was calculated[3b] to yield a pH of 7·6 with the indicated $NaHCO_3$ concentration, in a gas phase of 5 per cent CO_2 and 95 per cent N_2, at 37° and 740 mm. The displacement of air in the reaction vessels with the above gas mixture was carried out by means of the gas exchange technique under reduced pressure.[3c] The reaction volume was 3·2 ml, with 0·6 ml substrate solution in the side arm and all other reaction components in the main compartment. Equilibration was initiated at −60 min, with readings every 5 min beginning at −25 min. Dumping was effected at 0 min, with the side arm being washed twice with the reaction mixture. The manometers were read at 5 min intervals during the reaction period 0 to +40 min. Each set of conditions was run in duplicate.

We expressed the rate as

$$V = \frac{(\mu\text{l. } CO_2 \text{ at 25 min}) - (\mu\text{l. } CO_2 \text{ at 10 min})}{15} \times 60$$

where V signifies μl. CO_2/h evolved within the reaction interval of +10 through +25 min during which the rate was linear.

The enzyme system used in the experiment was characterized kinetically with acetylcholine iodide as the substrate. A linear reciprocal Lineweaver–Burk plot[4] of the enzyme activity, at varying enzyme and substrate concentrations, was graphically evaluated as suggested by Dixon[5] and Reiner;[6] the Briggs–Haldane constants[6,7] (0·0068, 0·0073, 0·0076 and 0·0062) did not vary significantly with enzyme concentration and were within

* Propionylcholine iodide, Dajax Laboratories, Leominster, Mass.

† Acetylcholine iodide, Nutritional Biochemicals Corp., Cleveland, Ohio.

the range of variability for independent determinations in the system investigated.[8]

Discussion

The results obtained in isolated human plasma 'pseudo'-cholinesterase systems appear to be rather interesting (see Tables I and II). The enzymatic hydrolysis of propionylcholine iodide and DL-lactoylcholine iodide did not seem to differ at all at the indicated substrate concentration. The greater than ten-fold reduction in the rate of enzymatic hydrolysis due to the β-methyl group in the choline component of the DL-lactoyl-DL-(β-methylcholine) analogue was more or less expected, since DL-acetyl-β-methylcholine behaves similarly in 'pseudo'-cholinesterase systems. If one should adopt Wilson and Bergmann's,[9] Wilson's[10], and possibly Porter, Rydon, and Schofield's,[11] concept of the site of enzyme-substrate interaction in acetylcholinesterase and serum cholinesterase systems, the data could be interpreted in terms of steric hindrance effected by the β-methyl function of the substrate which is situated within the area of interaction. The fact that the rate of the enzymatic hydrolysis of DL-glyceroylcholine iodide was only one tenth that of DL-lactoylcholine iodide, seemed at first somewhat surprising, since the β-hydroxy substitution on the acid component of the choline ester was even farther removed from the site of the substrate molecule believed to be directly involved in the usual enzyme-substrate complex[9] than the α-hydroxyl function of the lactic acid ester. In searching for an explanation for these findings, we noted that the β-hydroxyl function in glyceroylcholine could lend itself readily to the formation of a six-membered chelate ring in which the β-hydroxyl hydrogen would form a hydrogen bond with the carbonyl keto oxygen (see Figure 1). Since Wilson and Bergmann's,[9] and Bergmann, Wilson and Nachmansohn's[12] data indicate that variations in the electronic characteristics of the carbonyl keto function have considerable effect upon enzyme-substrate interaction at the esteratic site, it is reasonable to suppose that the influence of hydrogen bonding upon the carbonyl keto function may reduce the enzyme affinity for DL-glyceroylcholine iodide.

There are several indications in the literature as to the stereospecificity of cholinesterases.[13, 14–16] L(+)-Lactoylcholine iodide

Table I. The behaviour of hydroxy-substituted propionylcholine derivatives in isolated human plasma cholinesterase systems

Compound	CO_2 Evolution, V_{25-10}*				
	Evaluated Substrate			Control (acetylcholine iodide)†	
	M	auto hydrolysis	enzymatic hydrolysis	M	enzymatic hydrolysis
DL-Lactoyl-DL-(β-methylcholine) iodide	$8 \cdot 11 \times 10^{-3}$	Not sig.	58	$8 \cdot 28 \times 10^{-3}$	294
DL-Glyceroylcholine iodide	$8 \cdot 22 \times 10^{-3}$	34	62	$8 \cdot 24 \times 10^{-3}$	268
DL-Lactoylcholine iodide	$8 \cdot 15 \times 10^{-3}$	56	638	$8 \cdot 26 \times 10^{-3}$	296
Propionylcholine iodide	$8 \cdot 15 \times 10^{-3}$	Not sig.	632	$8 \cdot 25 \times 10^{-3}$	306

* Calculated μl. CO_2/h from rate during reaction interval of +10 through +25 min, during which the rate was linear.
† The autohydrolysis of acetylcholine iodide within the reaction interval of +10 through +25 min was not significant enough to be included.

Table II. Comparative rates of hydrolysis of lactoylcholines in isolated human plasma cholinesterase systems

Compound	CO_2 Evolution, $V_{25\text{-}10}$†				
	Evaluated substrate			Control (acetylcholine iodide)‡	
	M	auto hydrolysis	enzymatic hydrolysis	M	enzymatic hydrolysis
*D(−)-Lactoylcholine iodide $[\alpha]_D^{24}+5{\cdot}26°$ ($c=17{\cdot}06$ in MeOH)	$8{\cdot}13\times10^{-3}$	52	498	$8{\cdot}34\times10^{-3}$	294
*L(+)-Lactoylcholine iodide $[\alpha]_D^{23}-5{\cdot}66°$ ($c=11{\cdot}90$ in MeOH)	$8{\cdot}22\times10^{-3}$	68	758	$8{\cdot}18\times10^{-3}$	284
DL-Lactoylcholine iodide	$8{\cdot}15\times10^{-3}$	56	638	$8{\cdot}26\times10^{-3}$	296

* Nomenclature based upon original lactic acid molecule.
† Calculated μl. CO_2/h from rate during reaction interval of +10 through +25 min, during which the rate was linear.
‡ The autohydrolysis of acetylcholine iodide within the reaction interval of +10 through +25 min was not significant enough to be included.

was found to be hydrolysed 1·5 times faster than its enantiomer. Although the difference in the enzyme affinity for the two enantiomers is significant, it is not very great; at least not at this substrate concentration. It is conceivable that the isomeric ratio may be greater at lower substrate concentrations; this possibility is being investigated. One could also rationalize that the functions affected by the asymmetric centre are not within the actual

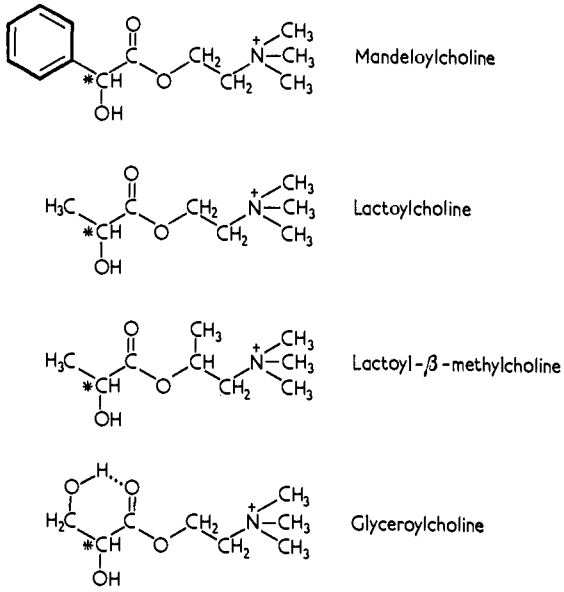

Fig. 1.

region of the substrate believed to be participating in the enzyme-substrate interaction[9] (see Figure 1), and that the volume of the affected functional groups is relatively small to induce major steric obstacles in this location.

Ammon and Meyer[16] reported greater substrate stereospecificity for mandeloylcholine in experiments with diluted human serum (isomeric ratios, 4 : 1 to 6 : 1 for D and L, respectively) during a reaction period of 18 h, at pH 5·4 and 37°; the control substrate, acetylcholine, was hydrolysed 2·5 times faster than DL-mandeloylcholine under these conditions. Notwithstanding the difference

in the nature of their experiments and those cited in this communication, it is quite obvious (see Figure 1) that the volume of the functional groups affected by the asymmetry in the mandeloylcholine molecule is considerably larger than that in the lactoylcholine moiety.

The fact that propionylcholine happens to be one of the cholinesterase substrates isolated from animal tissue[17] lends additional emphasis to the evaluation of α-mono- and α,β-dihydroxypropionylcholines; furthermore, since lactic acid is one of the simplest and most common carboxylic acids occurring in natural biochemical reactions, the actual existence of a natural lactoylcholine cannot be entirely excluded.

The pharmacodynamic properties of the new hydroxy substituted propionylcholines have been determined by Rama Sastry, Pfeiffer and Lasslo.[2, 18]

Summary. The hydrolysis of D(−)-, L(+)- and DL-lactoylcholines, DL-lactoyl-DL-(β-methylcholine) and DL-glyceroylcholine was studied in isolated human plasma cholinesterase systems. Relationships were explored between the molecular constitution of the new hydroxy substituted propionylcholines and the biochemical response.

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References

[1] Rama Sastry, B. V., Lasslo, A. and Pfeiffer, C. C. *J. Org. Chem.* **23,** 2005 (1958)
[2] Rama Sastry, B. V., Pfeiffer, C. C. and Lasslo, A. In preparation
[3] Umbreit, W. W., Burris, R. H. and Stauffer, J. F. *Manometric Techniques.* 1957. Minneapolis, Minn.; Burgess Publishing Co.: (a) p. 149; (b) p. 25; (c) p. 71.
[4] Lineweaver, H. and Burk, D. *J. Amer. chem. Soc.* **56,** 658 (1934)
[5] Dixon, M. *Biochem. J.* **55,** 170 (1953)
[6] Reiner, J. M. *Behavior of the Enzyme Systems,* p. 28. 1959. Minneapolis Minn.; Burgess Publishing Co.
[7] Briggs, G. E. and Haldane, J. B. S. *Biochem. J.* **19,** 339 (1925)
[8] Reiner, J. M. Personal communication
[9] Wilson, I. B. and Bergmann, F. *J. biol. Chem.* **186,** 683 (1950)

[10] Wilson, I. B. *J. biol. Chem.* **208,** 123 (1954)
[11] Porter, G. R., Rydon, H. N., Schofield, J. A. *Nature, Lond.* **182,** 927 (1958)
[12] Bergmann, F., Wilson, I. B. and Nachmansohn, D. *J. biol. Chem.* **186,** 693 (1950)
[13] Glick, D. *J. biol. Chem.* **125,** 729 (1938)
[14] Hoskin, F. C. G. and Trick, G. S. *Canad. J. Biochem. Physiol.* **33,** 963 (1955)
[15] Augustinsson, K. B. and Isacschen, T. *Acta chem. scand.* **11,** 750 (1957)
[16] Ammon, R. and Meyer, H. *Hoppe-Seyl. Z.* **314,** 198 (1959)
[17] Banister, R. J., Whittaker, V. P. and Wijesundera, S. *J. Physiol.* **115,** 55P (1951)
[18] Rama Sastry, B. V., Pfeiffer, C. C. and Lasslo, A. *Abstracts Amer. Soc. Pharmacol. and Exptl. Therap.*, September 1959; p. 67