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THE SYNTHESIS OF SOME ANALOGUES OF BUTYRYLCHOLINE AND THEIR HYDROLYSIS BY A PURIFIED HORSE SERUM CHOLINESTERASE

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Abstract—The Michaelis constants and maximum reaction velocity values for butyrylcholine, acetoacetylcholine, β-hydroxybutyrylcholine and crotonylcholine were determined using purified horse serum cholinesterase.

The methods used for the synthesis of the above compounds are also described.

THE BIOLOGICAL function of Pseudocholinesterase (PCE) (acylcholine acylhydrolase-3.1.1.8) is still obscure. It has been suggested that the prime function of PCE may be to hydrolyse the various choline esters formed during metabolic processes.[1–3] The acylation of choline by choline acetylase can be achieved using several acyl coenzyme A derivatives, including butyryl coenzyme A, to give the corresponding choline esters,[4] and it is suggested[3] that this synthetic pathway may be linked with the fatty acid cycle, causing synthesis in the body of the choline esters of the acids of the fatty acid cycle, in particular butyrylcholine. Since butyrylcholine has a powerful nicotinic action[6] and also is the optimum substrate for PCE[5], the physiological function of PCE may be to remove such esters from the system.

Acetoacetylcholine, β-hydroxybutyrylcholine and crotonylcholine, were therefore prepared and their behaviour as substrates of PCE examined.

METHODS AND RESULTS

Preparation of substrates

Butyrylcholine iodide. Butyrylcholine iodide was prepared by the method of Kyi & Wilson,[7] (1957) and had a m.p. of 92°. (Kyi and Wilson quote 93–94°).

2-Dimethylaminoethylacetoacetate methiodide. 2-Dimethylaminoethylacetoacetate methiodide was prepared by a modification of the transesterification reaction described by Phillips.[8] Sodium metal (0·5 g) was dissolved in 2-dimethylaminoethanol (21 g), ethyl acetoacetate (50 ml) was added and the mixture heated at 130–135° for about

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2 hr until the calculated amount (19 ml) of ethanol had been collected using a Dean and Stark trap. 2-Dimethylaminoethylacetoacetate (17·4 g; b.p. 75–80° at 2·4 mm) was isolated from the reaction mixture, an aliquot (5 g) in acetone (20 ml) was treated with methyl iodide (6 ml) and the solid obtained was crystallized from ethanol–acetone to give 2-dimethylaminoethylacetoacetate methiodide (9 g), m.p. 126·5–128°. (Found: C, 34·4; H, 5·7; N, 4·4; equiv. 315. $C_9H_{18}INO_3$ requires C, 34·3; H, 5·7; N, 4·4; equiv. 315.) Infra red spectroscopy showed the presence of two carbonyl groups (ν, C = O = 1710 and 1745 cm^{-1}).

2-Dimethylaminoethyl-β-hydroxybutyrate methiodide. Sodium hydroxybutyrate monohydrate (7·2 g) was added to 2-dimethylaminoethanol (18 g) in dry benzene (100 ml) and the solution saturated with dry hydrogen chloride. Toluene sulphonic acid (0·5 g) was added, and the mixture heated at 100–120° until the calculated amount of water (1·0 ml) had been collected in a Dean and Stark trap. 2-Dimethylaminoethyl-β-hydroxybutyrate (3·3 g; b.p.75–84° at 1·6–1·8 mm) was isolated from the reaction mixture and an aliquot (2·0 g) in acetone (15 ml) treated with methyl iodide (4 ml). The solid product was crystallized from ethanol acetone to give 2-dimethylaminoethyl-β-hydroxybutyrate methiodide (3·0 g) m.p.102–103° (Found: C, 34·1; H, 6·2; N, 4·5; equiv. 318. $C_9H_{20}INO_3$ requires C, 34·1; H, 6·3; N, 4·4; equiv. 317). Infra red spectroscopy showed the presence of both hydroxyl and carbonyl groups.

Unsuccessful attempts were made to prepare this compound by reducing aceto-acetylcholine using, (i) lithium *N*-hydropyridyl aluminium hydride and, (ii) by hydrogenation using platinum oxide and palladium on charcoal as catalysts. Direct esterification was attempted using several solvent systems but this process was only successful when *p*-toluene sulphonic acid was included in the reaction mixture.

2-Dimethylaminoethyl crotonate methiodide. 2-Dimethylamino ethanol (5 g) was converted to the dry hydrochloride, crotonyl chloride (6·5 g) and chloroform saturated with dry hydrogen chloride (25 ml) were added and the mixture heated at 85–100° for 6 hr. 2-Dimethylaminoethyl crotonate (3·4 g) b.p. 94–96° at 13 mm, was isolated from the reaction mixture and an aliquot (3·4 g) in acetone (15 ml) treated with methyl iodide (4 ml). The solid obtained was crystallized from ethanol to give 2-dimethyl-aminoethyl crotonate methiodide (5·2 g), m.p. 130°. (Found: C, 36·1; H, 6·0; N, 4·6. $C_9H_{18}INO_2$ requires C, 36·1; H, 6·0; N, 4·7).

Hydrolysis studies

The hydrolysis studies were conducted at 37° using the standard Warburg manometric technique.[9–11] The purified horse serum cholinesterase (PCE) was obtained by the method of Strelitz,[12] the procedure being followed to the end of Stage 5, the product freeze-dried over P_2O_5 and stored at 0°. The isolated enzyme was characterized using acetyl-β-methylcholine and the selective inhibitors Nu1250 and Nu683.[13, 14] The hydrolytic activity of a freshly-prepared enzyme solution was adjusted so that 1·5 ml hydrolysed approximately 2 mg of butyrylcholine at its optimum concentration in 30 min. A 1 in 66 dilution of the freeze-dried enzyme was prepared before use.

The results obtained from hydrolysis studies using the four compounds 2-dimethyl-aminoethyl butyrate methiodide (BuCh), 2-dimethylaminoethylacetoacetate methiodide (AcacCh), 2-dimethylaminoethyl-β-hydroxybutyrate methiodide (β-OHCh) and 2-dimethylaminoethylcrotonate methiodide (CrCh) are shown in Fig. 1. The information derived from these results is presented in Table 1.

DISCUSSION

The K_m value obtained for AcacCh appears to be at variance with the observed rate of hydrolysis relative to the other substrates studied (see Table 1). Since K_m values can be assumed to be inversely proportional to affinity values, the K_m values obtained for BuCh, β-OHCh and CrCh indicate that a β-hydroxy substituent results

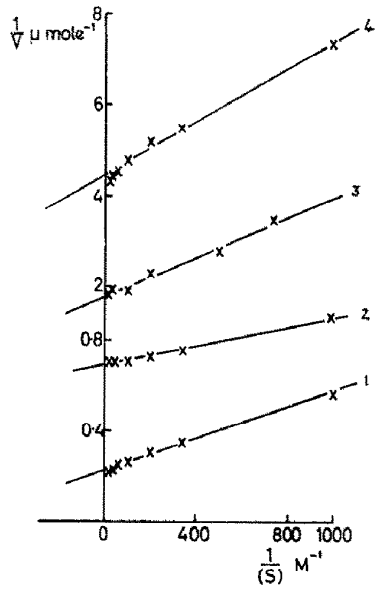

FIG. 1. The Relative rates of hydrolysis of some choline esters by serum cholinesterase; 1, BuCh; 2, AcacCh; 3, BOHCh; 4, CrCh.

TABLE 1. VALUES OF V_{max} AND K_m OBTAINED FOR BuCh, AcacCh, β-OHCh AND CrCh USING PCE

Substrate	V_{max} μmole/30 min	K_m M × 10^3
BuCh	4·44	1·52
AcacCh	1·45	0·29
β-OHCh	0·57	1·22
CrCh	0·22	0·76

Preliminary results* indicate that the pharmacological activities of AcacCh and β-OHCh are similar to the activity reported for CrCh,[17] and similar to, but less than, that reported for BuCh[6]; the compounds exhibit nicotinic activity but only weak muscarinic activity.

* W. C. Bowman, unpublished observations.

in an increased affinity of the substrate for the enzyme, and α-β unsaturation still more increases affinity. The increase is paralleled by a decrease in the reaction velocity (V_{max}). However, AcacCh appears to have a greater affinity than CrCh for the enzyme surface, but is hydrolysed more rapidly than β-OHCh. This can only be

explained by a high order of enzyme activity toward the substrate, which is probably enhanced by the susceptibility of the molecule to nucleophilic attack.

Exact estimates of the levels of aceto acetyl, β-hydroxybutyryl and crotonyl coenzyme A in the body are difficult to make. It is known[18] that large amounts of acetoacetic acid and β-hydroxybutyric acid can be produced in human and ruminant blood as end products in the process of β-oxidation of long chain fatty acids. Should the coenzyme A derivatives of these compounds react to form their choline derivatives, as is postulated, appreciable amounts of such choline derivatives would be formed and their accumulation would have undesirable and toxic effects. It can be calculated from the rates of hydrolysis given in Table 1 that the amount of enzyme present in horse serum can hydrolyse up to 0·1 μmole of CrCh/ml serum/min, and the other three compounds at a considerably faster rate. At extremely low substrate concentrations the rate of enzymic reaction is slow—only a proportion of the available enzyme being involved in metabolizing the substrate, cf. normal enzyme activity/substrate concentration rate curves—and therefore at the low *in vivo* concentration of these choline esters, a low K_m could be more important for their rapid hydrolysis than a high V_{max}. However, any of these compounds formed as metabolic by-products would be destroyed rapidly.

The work presented in this paper is consistent with the suggestion of Clitherow[3] that destruction of toxic metabolic by-products may be one of the primary physiological functions of serum cholinesterase.

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