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SHORT COMMUNICATIONS

Use of 1-methyl-acylquinolinium iodides for assaying acetylcholinesterase in tissue homogenates—Differentiation between acetylcholinesterase and cholinesterase

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MANY methods to assay acetylcholinesterase* (AChE; EC 3.1.1.7) making use of artificial substrates have been described. There are many enzymes acting on a carboxylic esterbond, and the problem arises to make an enzyme-specific substrate. Prince[1,2] chose 1-methyl-acetyoxyquinolinium iodides as AChE-substrates. The products of hydrolysis can be determined spectrophotometrically or fluorometrically (the latter only for the 7-acyl products).

We found that apart from AChE there are other enzymes that attack this type of substrate. We describe a routine assay procedure applicable to tissue homogenates, with the same specificity as AChE-assays in which acetylcholine is used as a substrate (Hestrin[3] and a modification of the radioactive procedure of Reed *et al.*[4]).

8-Acetoxy-1-methylquinolinium iodide (8-Ac-Q) was prepared by a modification of the method of Prince.[1]

All reactions were carried out at room temperature. During the acetylation we used dimethyl formamide as solute, recrystallizations were performed in ethanol.

8-*n*-Butyroxy-1-methylquinolinium (8-Bu-Q) was prepared with the use of *n*-butyric anhydride.

7-Acetoxy-1-methylquinolinum iodide (7-Ac-Q) was obtained in a similar way from 7-hydroxyquinoline (made according to the modified Skraup-reaction as described by Bradford *et al.*[5]) (for analytical data cf. Table 1). The non-enzymatic hydrolysis of the acyl-quinolinium compounds fit a first order kinetic at constant pH.

TABLE 1. ANALYTICAL DATA ABOUT THE ACYLQUINOLINIUM COMPOUNDS

Compound	Recrystallization from	% N		Melting point	Melting point Prince[1]
		Calculated	Found		
7-Ac-Q	Isopropanol	4·26	4·3; 4·3	217–220°	220–221°
8-Ac-Q	Ethanol	4·26	4·3; 4·2	184°	176·5–177°
8-Bu-Q	Isopropanol	3·92	3·9; 4·0	133–134°	—

The hydrolysis constants as a function of the pH are depicted in Fig. 1.

To arrive at a sensitive assay, this is performed at pH 6·0 as then at low enzyme activity the non-enzymatic hydrolysis is more reduced than the enzymatic one. The time of incubation is limited by the decrease of the substrate concentration. Circumstances are chosen so that less than 30 per cent of the substrate is hydrolysed (max. 20 min). Incubation volume is 1 ml, containing 10 μM phosphate buffer, pH 6·0, 15 μM $MgCl_2$, crude rat brain homogenate (containing AChE) and substrate. The reaction is stopped by adding 2·0 ml 5% TCA in methanol and the extinction or fluorescence was measured (Table2). The activity is linear with the time and the enzyme concentration in the range used.

Prince[1] studied the hydrolysis of quinolinium compounds by chymotrypsin and trypsin, and he found a very low activity. Yet we found[6] that these substrates are not enzyme-specific. Using AChE-inhibitors as eserine, DFP and Soman, it appeared impossible to inhibit more than 80 per cent of the enzymatic-hydrolysis by rat brain homogenates. Therefore we took the activity, sensitive to 10^{-5} M eserine (assayed in parallel series), as a measure of the acetylcholinesterase activity. We compared the pI-50 of the enzyme activity using different substrates (Table 3).

* Abbreviations used: AChE, acetylcholinesterase; 7-Ac-Q, 7-acetoxy-1-methylquinolinium iodide; 8-Ac-Q, 8-acetoxy-1-methylquinolinium iodide; 8-Bu-Q, 8-butyroxy-1-methylquinolinium iodide.

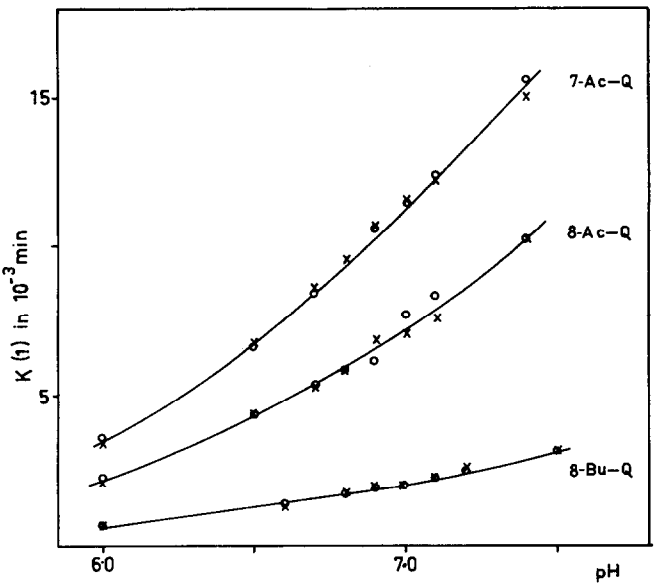

FIG. 1. Hydrolysis constants of acyl-1-methylquinolinium compounds.

TABLE 2. ASSAY CIRCUMSTANCES

Substrate	Concentration	Units AChE/ml	Assay method
7-Ac-Q	0·7 μM	0·0005–0·003	fluorometrically exc. 359 nm; emiss. 509 nm
8-Ac-Q	0·8 mM	0·005–0·02	spectrophotometrically 377 nm
8-Bu-Q	0·8 mM	—	spectrophotometrically 377 nm

TABLE 3. pI-50 OF AChE

Inhibitor	ACh as substrate	Quinolinium compound as substrate
Eserine	6·3	7·6 (8-Ac-Q)
DFP	7·1	7·1 (8-Ac-Q)
Soman	9·4	9·6 (7-Ac-Q)

The difference in pI-50 for eserine can be explained because it is a reversible competitive inhibitor and the pI-50 is a function of the affinity to the active site of the enzyme both of the inhibitor and of the substrate.

The pI-50 values for the irreversible inhibitors DFP and Soman for the two assays suggest that we are measuring the same enzyme.

To ascertain in a different way that the enzymatic hydrolysis of acetylcholine and quinolinium compounds is due to the same enzyme, we used the thermal denaturation. The time required to denature half of the enzyme-activity was determined by placing tubes, containing rat brain homogenates, in a 70° waterbath for different times. Subsequently the tubes were cooled in ice. The half life

value of the enzymatic activities with acetylcholine and quinolinium compound were 68 and 67 sec respectively, the denaturation curves were identical.

The localizations of the particle bound esterase activity over a continuous (0·8–1·7 M) sucrose gradient were very similar for both substrates.

8-Bu-Q was not hydrolysed faster in normal than in the eserinized rat brain homogenates. Apparently 8-Bu-Q is not detectably hydrolysed by AChE. In rat plasma, 8-Bu-Q is hydrolysed very quickly. This is an indication that it is possible to differentiate with this substrate between AChE and cholinesterase (EC 3.2.1.8) activity.

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Interaction of some daunomycin derivatives with deoxyribonucleic acid and their biological activity

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THE BIOLOGICAL activity of daunomycin is dependent on the ability to interact with primer DNA.[1]

In order to obtain further information about the mode of action of daunomycin, we studied some physicochemical properties of the complex which DNA forms with some daunomycin derivatives and their biological activity.

In the interaction of a chemical compound with biological material, the observed effects are in some cases highly specific, so that small changes in the molecule structure lead to marked changes in the biological activity.

We tested two groups of daunomycin derivatives. The derivatives of the first group have changes in acetyl side chain (R) in 9-position of the saturated ring of daunomycinone.

Among these the following were studied:

14-hydroxy-daunomycin (R = —CO.CH_2OH),
13-dihydro-daunomycin (R = —CHOH.CH_3),
daunomycin oxime (R = —CNOH.CH_3),
daunomycin semicarbazone (R = —CNNHCONH_2.CH_3),
daunomycin thiosemicarbazone (R = —CNNHCSNH_2.CH_3).

The derivatives of the second group have alterations in the amino-sugar (daunosamine). Among these are the following: *N*-acetyl-daunomycin, daunomycin-*N*-guanidine-acetamide, 2-amino-2-α-D-deoxy glucosyl-daunomycinone. A natural derivative of daunomycin with 2 moles of daunosamine