Heterogeneity of Smooth-Muscle Cholinesterase in Subcellular Fractions

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The differences in percentage inhibition by hexafluorenium bromide [hexamethylene-1,6-bis-(9-fluorenyldimethylammonium) dibromide (Mylaxa, Nessler, Mallinckrodt, Germany)] and in polyacrylamide-gel disc-electrophoretic pattern of the hydrolytic activity of acetylthiocholine iodide of different fractions of rat jejunum have been reported by Das & Sayer (1975). Scaf (1971) in his study on the effects of hexafluorenium on the cholinergic system of the gut used jejunum from both rat and guinea pig. Since, it is well known that the heterogeneity of enzymes is species-related, the present communication presents further results on properties and distribution of cholinesterases in guinea-pig jejunum.

Materials and methods

Laboratory animals, similar to those used by Scaf (1971), were used. All the methods were similar to those of Das & Sayer (1975), with the following exceptions:

(1) The dissected gut was first washed *in vivo* under slight pressure with ice-cold 200 mM-Sorenson's buffer, pH 7.4, containing 250 mM-sucrose and then removed to a beaker filled with the same buffer mixture. The gut was next washed inside out and the thin membrane coated with blood vessels was carefully stripped off and laid on a watch-glass at 0–4°C. Excess of luminal fluid was expelled and discarded. The mucoss was removed by clamping one end of the gut and gently stroking with the edge of a thin glass miroscope slide. Sections of mucosa-free organ were subsequently homogenized and fractionated.

(2) Tissue homogenizations, fractionations and solubilizations were carried out in 20 mM- instead of 67 mM-phosphate buffer, pH 7.4, containing 250 mM-sucrose. All separations were carried out at 0–4°C in Sorval RC-2 and Beckman Spinco L2 65B centrifuges. Designation of fractions, e.g. N, M, m and S, was as described by Das & Sayer (1975).

(3) Cholinesterase activities (μmol/min per g of wet tissue) and percentage inhibition values were measured as a routine by the coupled assay, with 5,5′-dithiobis-(2-nitrobenzoic acid) as the chromogenic reagent and acetylthiocholine, propionylthiocholine and butyrylthiocholine iodides as substrates, at 1mM concentration.

Table 1. *Characteristics of thiocholinesterase activity in different fractions of guinea-pig jejunum*

Percentage inhibition was determined in the presence of (*a*) eserine sulphate, (*b*) 1,5-bis-(4-allyldimethyl)ammonium bromide, (*c*) tetraisopropylpyrophosphate and (*d*) hexfluorenium bromide. The concentration of inhibitor was 5 μM in each case.

	K_m(μM)			Inhibition (%)			
Fractions	Acetyl-thiocholine	Propionyl-thiocholine	Butyryl-thiocholine	(*a*)	(*b*)	(*c*)	(*d*)
1N10	67	67	120	88	35	70	55
15M15	55	55	180	85	38	60	50
145m60	50	55	140	88	30	72	42
S	40	36	24	90	5	90	90

(4) In one experiment, cholinesterase assay was also performed with 2,2-dithiopyridine (Adrethiol-2; Sigma Chemical Co., St. Louis, MO, U.S.A.) as an alternative chromogenic reagent. All the routine assays were performed spectrophotometrically in a Unicam SP.8000 recording spectrophotometer at 25°C. The method of Brownson & Watts (1973) was used.

(5) The percentage-inhibition values were determined in the presence of the following inhibitors: (*a*) tetraisopropylpyrophosphate (a specific pseudocholinesterase inhibitor); (b) 1,5-bis-(4-allyldimethyl)ammonium bromide (a specific true cholinesterase inhibitor); (*c*) hexfluorenium bromide; (*d*) eserine sulphate and acetylthiocholine (1 mM) was used as substrate.

(6) In addition, inactivation of enzymes at different temperatures was measured by incubating the enzyme samples at defined temperatures for 15 min and subsequently cooling them at 4°C before activity measurement. Percentage remaining activity in each sample relative to that in the sample kept at 4°C was calculated. Similarly, the effect of pH on denaturation was investigated by maintaining the enzyme samples in different buffers of different pH values. The activities in each sample were compared with that of the extract maintained at pH 7.4 and 4°C. The optimum pH for the reaction for the enzymes in different fractions was also determined; a correction for the molar-absorption coefficient of the 5-thio-2-nitrobenzoate ion at various pH values was applied.

Results and discussion

Subcellular distribution of all thiocholinesterase activities similar to those described by Das & Sayer (1975) was observed. With all three substrates, the S fraction contained the most activity. Substrate specificity for all the particulate fractions, in order of effectiveness was propionylthiocholine $\simeq$ acetylthiocholine > butyrylthiocholine, whereas that for the S fraction was butyrylthiocholine $\simeq$ propionylthiocholine > acetylthiocholine. With the crude homogenates, it appeared that smooth-muscle cholinesterase preferably hydrolysed propionylthiocholine, and most of this activity was pseudocholinesterase. Moreover, particulate fractions contained both pseudocholinesterase and true cholinesterase, whereas the S fraction contained only the pseudocholinesterase type. This is also evident from the inhibition studies, as shown in Table 1. First, an equally preferred affinity of particulate cholinesterases for acetylthiocholine and propionylthiocholine was observed, whereas the S fraction showed preferred affinity for butyrylthiocholine, as judged by the respective K_m values. Secondly, tetraisopropylpyrophosphate inhibited almost totally all the activities of the S fraction, and 1,5-bis-(4-allyldimethyl)ammonium bromide had no effect. On the other hand, particulate cholinesterase was inhibited by 60–70% and 30–40% by tetraisopropylpyrophosphate and 1,5-bis-(4-allyldimethyl)ammonium bromide respectively. In other words, it appears that the particulate fraction contains about 30–40% of true cholinesterase and the rest is pseudocholinesterase.

Differences in temperature- and pH-stability and in pH profile were also observed between the particulate-bound and the soluble cholinesterase.

Five electrophoretically distinct subcomponents of cholinesterase have been identified in different subcellular fractions. The electrophoretic profile, (polyacrylamide-gel disc electrophoresis) of cholinesterases in different fractions also appeared to be different.

The results of the present investigation together with those of previous reports (Das & Sayer, 1975) demonstrate that smooth-muscle cholinesterases are heterogeneous with regard to their substrate and inhibitor specificity, temperature and pH effects and electrophoretic pattern.

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