THE INHIBITORY EFFECT OF PROPRANOLOL AND ISOPROTERENOL ON HUMAN PLASMA CHOLINESTERASE

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ABSTRACT

The effect of propranolol and isoproterenol on the hydrolysis of 4- nitrophenylbutyrate (PNPB) by the purified human plasma cholinesterase was studied. During the hydrolysis of PNPB, enzyme obeyed to Michaelis-Menten model. Propranolol was found to be a competitive inhibitor, and isoproterenol yielded a complex inhibition pattern. It could be explained that the inhibitory effect of propranolol shows noncooperativity between subunits of human plasma cholinesterase upon binding of PNPB. In contrast, isoproternol inhibitory effects indicate more than one type of binding sites on this enzyme.

Key words: Human plasma cholinesterase, Propranolol, Isoproterenol, kinetic, Asymmetry.

INTROUCTION

Cholinesterase (ChE) or butyrylcholinesterase (BChE;acylcholine acyl hydrolase: EC 3.1.1.8) is a serine hydrolase which has the ability to hydrolyze a wide variety of esters such as acyl or benzoylcholine and butyrylthiocholine.

Based on the substrate specificity and tissue distribution, the enzyme is divided into two groups: acetylcholinesterase and cholinesterase (1,2). Acetylcholinesterase is specific for acetylcholine as substrate but cholinesterase, which is found in plasma, hydrolyzes non choline as well as choline esters of a variety of carboxylic acid (2,3). The CHE has a tetrameric structure with four identical subunits (4).

Cholinesterases are targets for organophosphorus compounds which are used as insecticides, chemical warfare agents and drugs for the treatment of diseases such as glaucoma, or parasitic infections(5-7). Many drugs of therapeutic importance, such as hexaflavorenium, pancuronium, succinylcholine, propranolol are reversible inhibitors of the enzyme(5,7,8-10). There are various reports about kinetic behaviour of horse cholinesterase toward inhibition by propranolol and some other inhibitors (11-14). Also it has been reported that the type of substrates affect inhibition characteristics (8,15,16).

In this study it was aimed to find kinetic behaviour of human plasma Cholinesterase toward propranolol and isoproterenol as inhibitors, using paranitrophenylbutyrate as the substrate.

MATERIALS AND METHODS

Materials

All chemicals and CH-Sepharose4B were from Sigma Chemical Company (UK). DEAE-tris-acrylamide was a product of LKB (Sweden). Blood was obtained from a single donor and used directly. 4-nitrophenylbutryate was dissolved in acetonitrile and other compounds were dissolved in distilled water.

Purification

Human plasma cholinesterase was purified from 130 ml of plasma of a single donor by the reported two steps purification method (17). The plasma was first dialyzed against one liter of 25 mM sodium acetate (pH 4.5) containing 1mM mercaptoethanol and 1mM EDTA. The dialyzed cholinesterase was applied to DEAE-tris-acrylamide anion exchange column (2.5õ35 cm) which had already been equilibrated with the same buffer. The enzyme was eluted with a total volume of 1200 ml of 0 to 0.5 M linear NaCl gradient. The active fractions were collected and dialyzed against buffer containing 20.0 mM potassium phosphate and 1mM EDTA (pH 7.2). The dialyzed sample was applied onto a procainamide-sepharose 4B column (0.9 õ 23.0cm) which was equilibrated with 20.0 mM potassium dihydrogen phosphate buffer cantaining 1 mM EDTA. The column was washed with dialyzed buffer plus 0.1M NaCl, until the liquid that was eluted from the column showed no absorption at 280 nm. Cholinesterase was eluted using a linear gradient of NaCl from 0.1 to 0.6M, in 150 ml of the phosphate buffer (figuer 1). Active fractions were collected and concentrated by ultra-filtration (Amicon PM 10). The criteria of purity of human plasma ChE was its specific activity of 530 ìmole.min^{-1}. ml^{-1}. mg^{-1} protein. Purfied enzyme was stored at-20 C.

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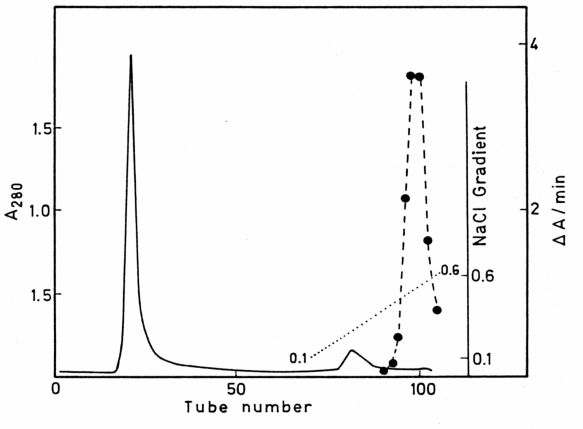

Fig 1. Plots of human plasma ChE purification by affinity chromatography. The affinity matrix was procainamid-sepharose 4B; Flow rate, 20 ml/hr. Absorbance at 280nm (—), Activity measurment, A/min (- -), NaCl gradient (...).

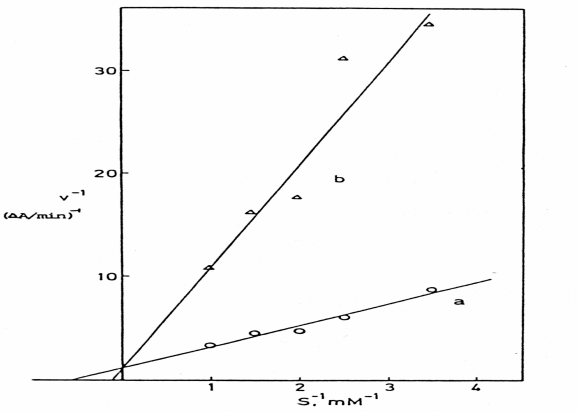

Fig 2. Lineweaver -Burk plot of PNPB hydrolysis and Propranolol effects on this hydrolysis.S=PNPB, PNPB hydrolysis (-o-),PNPB plus 0.015mM propranalol (- -).

Activity measurment

Human plasma ChE activity was measured by colorimetric method as described by Ellman et al (18). The assay mixture contained in final volume of 1ml: 100mM potassium phosphate buffer (pH 7.4) : 5mM of 2-mercaptoethanol and different concentration of PNPB as substrate. The reaction was initiated by the addition of 20 ìl (0.8 ìg) of the pure cholinesterase enzyme. The mixture was incubated at 30 C. The reae tion was followed by the measurment of the absorbance value at 405 nm.

RESULTS

1. Effect of propranolol on PNPB hydrolysis

Lineweaver-Burk plot and Dixon plot for the hydrolysis of PNPB by human plasma ChE in the presence of propranolol are given in figures 2 and 3 respectively.From the Lineweaver-Burk plot Vmax, Km and Ki values were found to be 0.83, 1.82 and 3.8 õ 10^{-3} mM/min, respectivity. Since the Vmax values are identical in the presence and absence of inhibitor, it appears that propranolol is a competitive type of inhibitor by PNPB hydrolysis. The Dixon plot, for the inhibition of PNPB hydrolysis,by propranolol at two different fixed concentration of substrate (0.2 and 0.5 mM) is linear (Figure3). The intersection point of the lines on Vmax $^{-1}$ represents also a competitive type of inhibition.The Ki obtained from the Dixon plot is 4.0 õ 10^{-3} mM.

2. Effect of isproterenol on PNPB hydrolysis

The inhibition of the hydrolysis of PNPB by isoproterenol gave a complex inhibition pattern. A non linear Dixon plot at two fixed concentration of substrate (0.2, 0.5 mM), are shown in figure 4. It seems that both km and Vmax are affected..Complex inhibition pattern of this inhibitor on PNPB hydrolysis could only be explained by the existence of multiple binding sites on the enzyme.

DISCUSSION

There are a number of studies for determination of kinetic parameters and recognition of kinetic properties of the enzyme in the presence of different inhibitors (6, 9, 11- 14). There are few investigations about the kinetic behaviour of horse plasma cholinesterase. Soylemez et al found that the kinetic behavior of inhibition of horse plasma cholinesterase by propranolol depends to the nature of the substrate. With butyrylthiochoine as substrate, a first–order kinetic, and with 4-nitrophenylbutyrate as substrate, a biphasic relationship was obtained (16,19). Ozer et al (8) have reported that human plasma cholinesterase was inhibited by carbodimide in a biphasic manner when butyrylthiocholine was used as substrate.

In order to resolve the kinetic behaviour of the human plasma ChE, the effect of inhibitors such as propranolol and isoproterenol on the hydrolysis of substrate (PNPB) were studied. The straight lines(Lineweaver-Burk and Dixon plot) in figures 2 and 3 indicates that propranolol is a competitive inhibitor of human plasma ChE with respect to PNPB as substrate. From linearity of the graph, it can be predicted that there are not any cooperativity between subunits of

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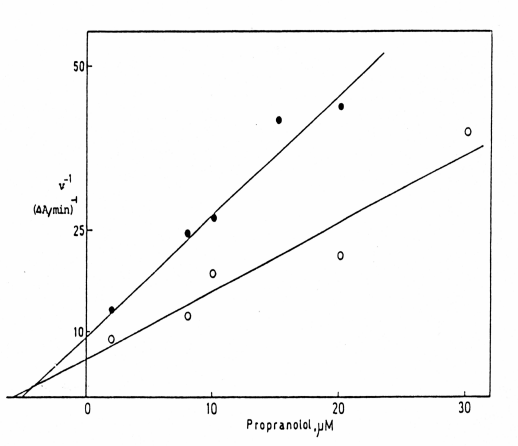

Fig 3. Dixon Plots of propranolol inhibitory effect on PNPB hydrolysis. 0.2 nM PNPB(-•-), 0.5 mM PNPB (-o-).

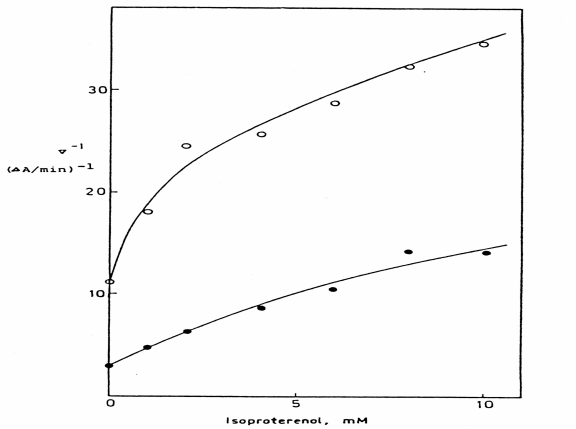

Fig 4. Dixon plots of isoproterenol inhibitory effect on PNPB hydrolysis. 0.5 mM PNPB (-•-), 0.2mM PNPB (-o-).

human plasma ChE upon binding to PNPB. From the non linear Dixon plot (Figure 4) for inhibitory effect of isoproterenol on PNPB hydrolysis by human plasma cholinesterase, it can be suggested that there must be more than one types of sites on human plasma cholinesterase which shows complex kinetic behaviour. By these information, it could be suggested that human plasma cholinesterase might be functionally asymmetric with respect to certain effectors and presently it is not clear whether this asymmetric function is an inherent property of the human plasma enzyme or it is induced by association with ligands (substrate). Further kinetic analysis are required to resolve these issues.

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