

THE INHIBITORY EFFECT OF PROPRANOLOL AND ISOPROTERENOL ON HUMAN PLASMA CHOLINESTERASE

ALI AWSAT MELLATI*, JAVAD TAJKEY**

* Department of Biochemistry, Zanjan University of Medical Sciences

** Department of Pharmacology, Zanjan University of Medical sciences, Zanjan, Iran

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, isoproterenol inhibitory effects indicate more than one type of binding sites on this enzyme.

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

INTRODUCTION

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-Sepharose 4B 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-nitrophenylbutyrate 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 (2.5×35 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 × 23.0cm) which was equilibrated with 20.0 mM potassium dihydrogen phosphate buffer containing 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 (figure 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 $\mu\text{mole} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein. Purified enzyme was stored at -20 °C.

Correspondance: Ali Awsat Mellati, Department of Biochemistry, Zanjan University of Medical Sciences, Zanjan, Iran, Email mellati.a.a@wowmail.com

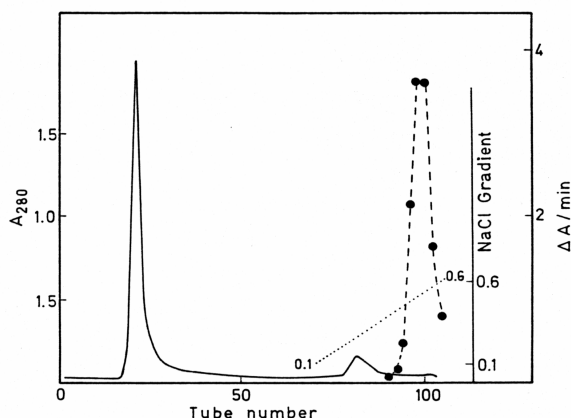


Fig 1. Plots of human plasma ChE purification by affinity chromatography. The affinity matrix was procainamid-sepharose 4B; Flow rate, 20 ml/hr. Absorbance at 280nm (—), Activity measurement, A/min (---), NaCl gradient (...).

Activity measurement

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 μ l (0.8 ig) of the pure cholinesterase enzyme. The mixture was incubated at 30 °C. The reaction was followed by the measurement 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 V_{max} , K_m and K_i values were found to be 0.83, 1.82 and 3.8×10^{-3} mM/min, respectively. Since the V_{max} 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 V_{max}^{-1} represents also a competitive type of inhibition. The K_i obtained from the Dixon plot is 4.0×10^{-3} mM.

2. Effect of isoproterenol on PNPB hydrolysis

The inhibition of the hydrolysis of PNPB by

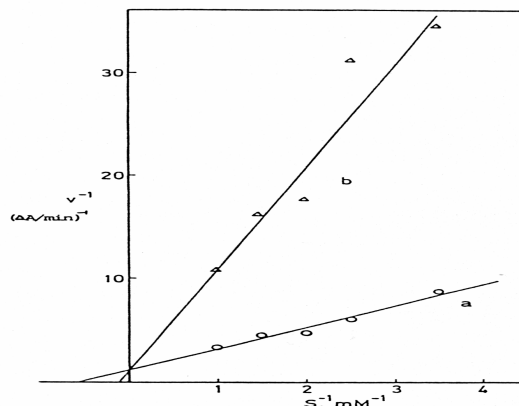


Fig 2. Lineweaver -Burk plot of PNPB hydrolysis and Propranolol effects on this hydrolysis. S=PNPB, PNPB hydrolysis (Δ), PNPB plus 0.015mM propranolol (\circ), PNPB plus 0.03mM propranolol (\square).

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 K_m and V_{max} 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 butyrylthiocholine 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

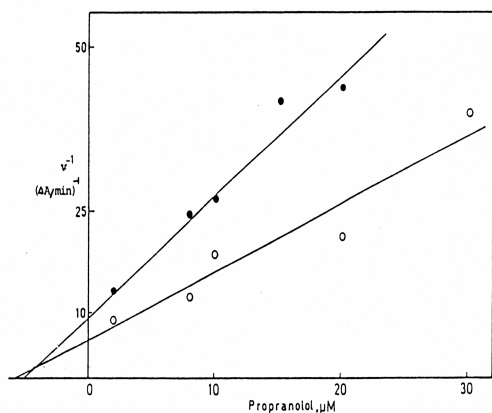


Fig 3. Dixon Plots of propranolol inhibitory effect on PNPB hydrolysis. 0.2 nM PNPB (●-), 0.5 mM PNPB (○-).

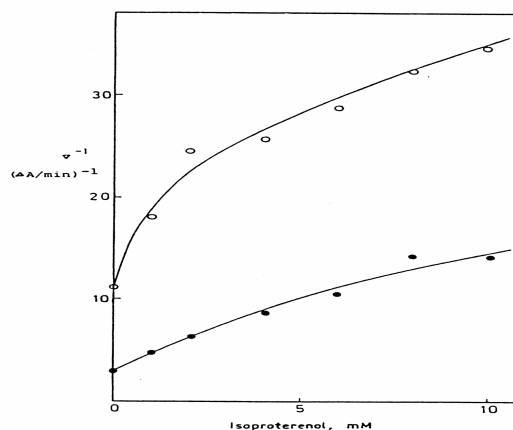


Fig 4. Dixon plots of isoproterenol inhibitory effect on PNPB hydrolysis. 0.5 mM PNPB (●-), 0.2mM PNPB (○-).

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.

REFERENCES

1. Brown, S.S., Kalow, W., Pilz, W., Whittaker, M., Woronick, C.L. (1988) The plasma cholinesterase; a new perspective. *Adv. Clin. Chem.* 22: 1-123
2. Ortonne, J.P., Voulot, C., Menezo, O. (1979) Cholinesterase isoenzymes: a comparative study in the skin and plasma. *J. Invest. Dermatol.* 73: 239-242.
3. Wilson, I.B. (1954) The active surface of the serum esterase. *J. Biol. Chem.* 208: 123-132
4. Lockridge, O. (1979) Interchain disulfide bonds and subunits organization in human serum cholinesterase. *J. Biol. Chem.* 254: 8234-8330.
5. Bodur, E., Cokugras, A.N., Tezcan, E.F. (2001) Inhibition effects of benzactyzine and drofenine on human serum butyrylcholinesterase. *Arch. Biochem. Biophys.* 386: 25-9.
6. Lockridge, O., Masson, P. (2000) Pesticides and susceptible populations: People with butyrylcholinesterase genetic variants may be at risk. *Neurotoxicology.* 21: 113-26.
7. Amitai, G., Moorad, D., Adani, R., Doctor, B.P. (1998) Inhibition of acetylcholinesterase and butyrylcholinesterase by chlorpyrifos-oxon. *Biochem. Pharmacol.* 56: 293-9.
8. Ozer, N., Ozer, I. (1987) Differential reactivity of active sites in human plasma cholinesterase to ward 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. *Arch. Biochem. Pharmacol.* 255: 89-94.
9. Perez Guillermo, F., Martinez pretel, C.M., (1988) Prolonged suxamethonium-induced neuromuscular blockade associated with organophosphate poisoning. *Br. J. Anaesth.* 61: 233-6
10. Cokugras, A.N., Tezcan, E.F. (1997) Amitriptyline: a potent inhibitor of butyrylcholinesterase from human serum. *Gen. Pharmacol.* 29: 835-8
11. Whithiker, M., Britten, J.J., Wicks, R.J. (1981) Inhibition of the plasma cholinesterase variants by propranolol. *Br. J. Anaesth.* 53: 511-6
12. Zaman, Z., Speeleveld, E., Sneyers, L., Desmet, K. (1997) Inhibition of acetylcholine esterase and choline esterase by benzethonium chloride and avoidance of the benzethonium chloride carry-over inhibitory effect. *Eur. J. Clin. Chem. Clin. Biochem.* 35: 603-7
13. Graham, S.G., Crossley, A.W. (1995) The characteristics of the inhibition of serum cholinesterase by metoclopramide. *Eur. J. Clin. Pharmacol.* 48: 225-8

Correspondance: Ali Awsat Mellati, Department of Biochemistry, Zanjan University of Medical Sciences, Zanjan, Iran, Email mellati.a.a@wowmail.com

14. Leader, H., Vincze, A., (1999) Characterization of O,O-dietylphosphoryl oximes as inhibitor of cholinesterases and substrates of phosphotriesterases. *Biochem. Pharmacol.* 58: 503-15
15. Whittaker, M., Wicks, R.J., Britten J.J. (1982) Effect of propranolol on human plasma cholinesterase when benzoylcholine was used as substrate. *Clin.chim. Acta.* 119:107-114
16. Soylemez, Z., Ozer, I. (1984) Substrate-dependent kinetic behaviour of horse plasma cholinesterase: evidence for kinetically distinct populations of active sites. *Arch. Biochem. Biophys.* 235(2): 650-6
17. Lockridge, O., LaDu, B.N. (1978) Comparison of atypical and usual human serum cholinesterase. *J.Biol.Chem.* 253: 361-366
18. Ellman, G.L., Courtery, D.D., Andres, V., Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7: 88-95
19. Erikson, H., Augustinsson, K.B. (1979) A mechanistic model for butyrylcholinesterase. *Biochim. Biophys. Acta.* 567: 61-173

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