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Hydrophobic Interaction Between the Acyl Moiety of Choline Esters and the Active Site of Acetylcholinesterase

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Abstract: Existence of a binding site for choline esters with an acyl chain of various sizes was examined by comparing the inhibitory potency of the respective compound. In contrast to acetylcholine, which showed a pure competitive pattern of inhibition, choline esters with an acyl chain of a long size ($C \geq 5$) expressed a mixed type of inhibition. Binding of choline esters containing a long chain (C_7-C_{12}) to the hydrophobic region in the active site is deduced from a linear relationship between the K_{iE} value and the size of acyl moiety, and a good hydrophobicity relationship. In addition, the non-competitive component in the inhibition of acetylcholinesterase seems to be due to the interaction of choline esters with both the hydrophobic site and the trimethylammonium-binding site in the active center of the acetylated acetylcholinesterase.
Key words: acetylcholinesterase, active site, choline esters, hydrophobic binding.

Earlier kinetic studies (Quinn, 1987; Sussman *et al.*, 1991) indicated that the active site of acetylcholinesterase contained two subsites, esteratic site and anionic site, corresponding to the catalytic machinery and the choline-binding pocket, respectively. The concept of an anionic subsite has been widely accepted since the early 1950s. Since the potency of ammonium compounds as inhibitors of the enzyme increases with the number of methyl groups, it is evident that the anionic subsite is not only anionic but also hydrophobic.

A recent X-ray study (Sussman *et al.*, 1991; Schafferman *et al.*, 1992) revealed that a 20 Å deep and narrow active site gorge containing the catalytic triad is lined by 14 aromatic amino acids, such as tryptophan and phenylalanine residues. In addition, a mutagenesis study indicates that the subsite specificity of acetylcholinesterase is determined by phenylalanine residue in the active center (Ordentlich *et al.*, 1993). Moreover, the study of the structure-activity relationship revealed that the interaction of choline esters with the active site of carbamoylated-acetylcholinesterase is dependent on the size of acyl moiety (Kim *et al.*, 1992; Sok *et al.*, 1994). However, a kinetic analysis on the interaction of choline esters with the active site was not performed in detail, although tetra-alkylammonium salts had been proposed to bind to acetylated enzyme (EA).

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Here, we compare the K_{iE} value (competitive component) and the K_{iES} value (non-competitive component) of choline esters with acyl chain of various sizes, and attempt to examine the interaction of choline esters with the active site of acetylcholinesterase.

Materials and Methods

Materials

Various choline esters or thiocholine esters were from Sigma Chemical Co. (St. Louis, USA), except for the choline esters such as pentanoylcholine iodide, hexanoylcholine iodide, heptanoylcholine iodide, octanoylcholine iodide and decanoylcholine iodide, synthesized as previously described (Kim *et al.*, 1992).

Assay of acetylcholinesterase

AChE activity was assayed according to the Ellman method (1961). Standard assays were performed in 0.1 M sodium phosphate buffer, pH 7.4, containing 0.5 mM acetylthiochoine and 0.33 mM DTNB. The assay was carried out at 25℃, and the change of absorbance at 412 nm was monitored using a Gilford 250 spectrophotometer.

Preparation of soluble acetylcholinesterase

Mice (ICR, 25~30 g) were sacrificed by decapitation without anesthesia, and the whole brain was dissected out, rinsed in cold saline, and homogenized using Dy-

namix teflon-glass homogenizer in 5 volumes of 0.1 M sodium phosphate buffer, pH 7.4. The homogenate was then centrifuged at 170,000 g for 90 min at 4℃ and the pellet was rehomogenized with the same volume of 0.1 M phosphate buffer (pH 7.4) containing 1.0% Triton X100, and centrifuged again at 170,000 g for 2 h. The supernatant was the source of membrane-bound AChE (detergent-soluble, DS-AChE), which was chromatographed, on a DEAE-Sephacel ion exchange column as described (Cho *et al.*, 1994).

Determination of K_i values

For the inhibition of AChE activity, the appropriate choline ester was added to the assay mixture, and the inhibition constants, a competitive (K_{iE}) and a non-competitive (K_{iES}) component, were determined from double-reciprocal plots of reation velocity against substrate concentration (Wilson and Alexander, 1962).

Results and Discussion

The inhibitory effect of choline esters with acyl moiety of various sizes on brain acetylcholinesterase was investigated. While acetylcholine was observed to be a pure competitive inhibitor in this study, the other choline esters (C_4-C_{14}) appeared to show a mixed type of inhibition (Fig. 1). Especially, the inhibition by choline esters with acyl moiety of a long chain length (C_8-C_{14}) consisted of a competive component (K_{iE}) and a non-competitive one (K_{iES}).

When the K_i values of the respective choline ester were determined, the K_i values differed greatly according to the size of the acyl group in the structure of choline esters (Table 1). Choline esters with acyl chain of a short (C_2-C_3) or an intermediate (C_5-C_6) size showed a similar inhibition of acetylcholinesterase with K_i values ranging from 62 μM to 74 μM. Noteworthy, butyrylcholine (C_4) expressed a higher inhibitory potency with a K_{iE} Value of 23μM, which is close to that (K_i, 32 μM) of heptanoylcholine. This might be explained by the assumption that the active center excludes the choline esters with an acyl chain longer than butyryl moiety. Thus the binding site for butyryl moiety of butyrylcholine was supposed to be separate from the site for a heptanoyl chain of heptanoylcholine, although both binding sites are suggested to be of a hydrophobic character. It is interesting to observe that among choline esters with a short chain length (C_2-C_4), the inhibitory potency was dependent on the length of acyl chain. The hydrophobic binding of choline esters (C_7-C_{12}) to the active site may be supposed from the observation that the inhibitory potency of choline esters with a long acyl chain was enhanced with increasing length of acyl chain.

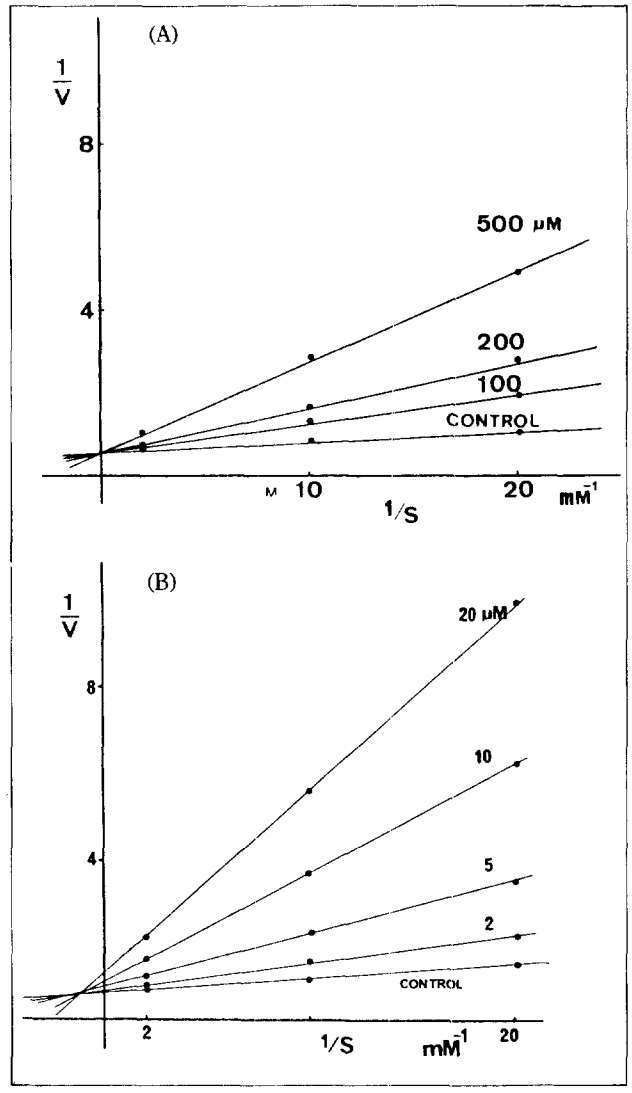

Fig. 1. Lineweaver-Burk double reciprocal plot for the inhibition of acetylcholinesterase by choline ester. The enzymatic hydrolysis of acetylthiocholine was determined in the presence of acetylcholine (100~500 μM) or myristoycholine (2~20 μM) in 0.1 M phosphate buffer (pH 7.4) containing acetylthiocholine (50~500 μM). (A) acetylcholine; (B) myristorylcholine.

To show a ligand selectivity in the binding of these choline esters (C_7-C_{14}) to a hydrophobic region in the active site, the log K_{iE} and log K_{iES} values were compared with the chain length of an acyl group corresponding to a hydrophobicity. Fig. 2 shows that the inhibitory potency of choline esters increased proportionally according to the length of the acyl chain. However, such a relationship was not observed with choline esters containing acyl chains either shorter than the heptanoyl group or longer than the lauryl moiety. The linear plot of log K_{iE} or log K_{iES} values against the hydrophobicity of an acyl chain demonstrates the hydrophobic nature of the inhibitor binding with the active

Table 1. K_{iE} and K_{iES} values for choline esters

No.	Compound	K_{iE} values (μM)	K_{iES} values (μM)
1	Acetylcholine	62.3± 9.9	–
2	Propionylcholine	66.7± 1.8	–
3	Butyrylcholine	23.8± 1.44	–
4	Pentanoylcholine	72.3± 0.52	1,100± 150
5	Hexanoylcholine	73.7± 1.39	1,050± 212
6	Heptanoylcholine	32.4± 4.94	730± 99
7	Octanoylcholine	16.6± 0.83	425± 22
8	Decanoylcholine	5.2± 0.18	113± 18
9	Laurylcholine	1.8± 0.02	25± 3
10	Myristoylcholine	1.8± 0.06	22± 3

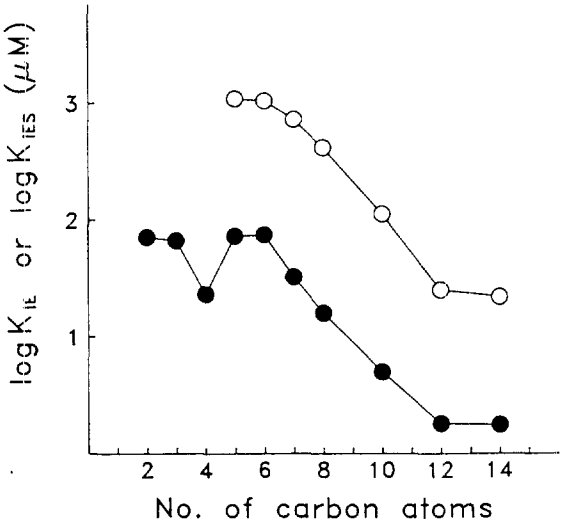

Fig. 2. Relationship between the inhibitory potency and the size of acyl group in choline esters. The enzymatic hydrolysis of acetylthiocholine was determined in the presence of each choline ester, and K_{iE} (competitive) and K_{iES} (noncompetitive) were determined as described in Experimental section. ●–●: K_{iE}. ○–○: K_{iES}.

site of free enzyme (E), ES form or EA form. As shown in Fig. 2, the binding site is characterized by slope= 0.44 for the K_{iE} value, and slope=0.64 for the K_{iES} value, based on $\pi CH_2=\pi CH_3$ (Leo *et al.*, 1971). Thus, the hydrophobic binding ($K_i=40.43\pi-5.97$ MR+69.32 (n=6, r=0.904, $r^2=0.817$)) of choline esters (C_7-C_{12}) in the active site can be regarded as a simple partition equilibrium between hydrophobic inhibitors and a hydrophobic region (unpublished data). In addition, it appears that a hydrophobicity is more important than a steric effect. Moreover, since the slope value for the K_{iES} value was greater than that for the K_{iE} value, it was supposed that the hydrophobic interaction in the binding of choline esters to active site is more contributory in ES or EA form than the free enzyme (E). This might explain why choline esters with chain lengths of a longer size show a greater non-competitive component in the inhibition of acetylcholinesterase. Therefore, it is supposed that the interaction of acyl moiety (C_7-C_{12}) with the hydrophobic region may lead to the formation of a non-competitive component (K_{iES} value) by interfering with the dissociation of ES or EA form. It is more likely that choline esters may interact with EA form rather than ES complex, since the site for trimethylammonium moiety is not available in ES complex. This might be consistent with a previous report (Wilson and Alexander, 1962) that the non-competitive component in the inhibition of acetylcholinesterase by quaternary ammonium salt containing a long alkyl chain may be due to its association with the hydrophobic region of acetylated enzyme (EA). Although the width of the hydrophobic region was not investigated, a limited width is suggested from the reports that the active site accommodates acridinium salt (Quinn, 1987), but not larger compounds such as propidium and gallamine (Barak *et al.*, 1994).

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