J. Pharm. Pharmac., 1975, 27, 855

COMMUNICATIONS

A modified structure for the acetylcholinesterase receptor

Recent results using substrates and inhibitors based upon rigid molecules with appropriate groups located on the bornane and bicyclo (2,2,2) octane molecules, now lead us to propose a modification (see Fig. 1b) of the active site of acetylcholinesterase previously proposed (Fig. 1a) (Beckett, 1967).

The main changes are:—

(1) The anionic site is subdivided into three negatively changed areas with their accompanying hydrophobic regions. Two of these are considered to be essential to accommodate two methyl groups of the onium head while the third less important area accommodates the third methyl group of a trimethylammonium head or a hydrogen atom from a protonated tertiary basic head; this third area accounts for some tertiary amine inhibitors (i.e. with two methyl groups) being better inhibitors than their trimethylammonium analogues but does not play a major role in the accommodation of substrate to receptor.

The proposed change will facilitate the binding of the *N*-delocalized positive charge to the hydrogen atoms of the methyl groups [i.e. + 0·21 for 3 hydrogen atoms but only +0·06 charge on the nitrogen atom of the onium head (see Pullman, Courriere & Coubeils, 1971)]. The proposed change requires the definition and localization of the three negative areas of the receptor site as shown in Fig. 1b.

(2) The location of the methyl groups as above allows a more precise definition of the location of the serine OH group and the acid site of the receptor than was proposed earlier.

(3) The proposed position of the imidazole group in the receptor has been relocated to facilitate hydrogen bonding as shown in Fig. 2b and its distance from the serine OH group has been defined more precisely.

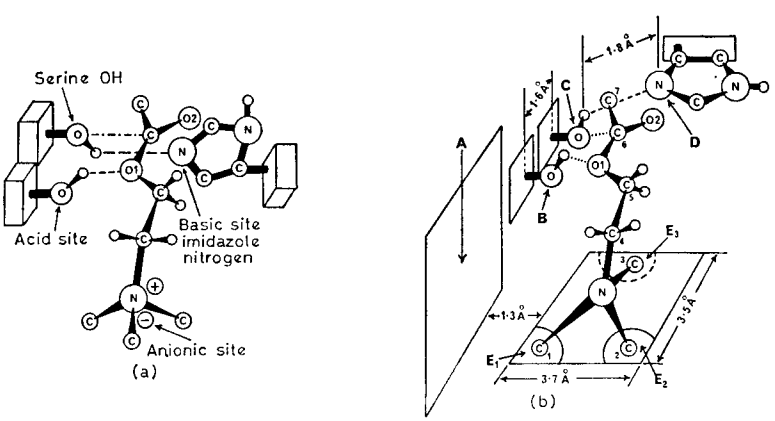

FIG. 1. Acetylcholine at the acetylcholinesterase receptor. (a) Receptor proposed in 1967 by Beckett. (b) New proposed receptor. A = Area of steric hindrance. B = Acid site (probably tyrosine OH). C = Serine OH. D = Histidine (imidazole) residue. E = Anionic site E_1 and E_2 electrostatic and hydrophobic anionic sites (specific). E_3 = Minor electrostatic and hydrophobic anionic site.

(4) A region of steric hindrance located in the receptor between the anionic sites and the esteratic site is proposed to account for differences in the inhibitory activities of the enantiomers of 3-*endo*-dimethylaminobornan-2-one methobromide; the proposed differences in fit of these two enantiomers is shown in Fig. 3.

It is proposed that the anionic sites first attract the onium head with its delocalized positive charge so that two methyl groups are correctly located at the receptor at E_1 and E_2 to facilitate ionic attraction reinforced by hydrophobic bonding. The location of a third methyl (or proton) at the third anionic site E_3 can assist this binding. Possibly an aspartic acid moiety is involved as E_1 and E_2 receptor sites as shown in Fig. 2a. This association between the cationic head of the substrate (or inhibitor) and the anionic site of the receptor is regarded as the orienting forces for primary molecular interaction. Subsequent orientation of the rest of the substrate or inhibitor molecule to the esteratic site depends upon the other structural features of the molecule and the nonbonded interaction which may result upon association of the oxygen functions with the esteratic site.

The newly proposed receptor makes explicable the following facts (*i–iv*) better than does the receptor previously suggested (Beckett 1967).

(*i*) The (+)-isomer (Ki $6{\cdot}7 \times 10^{-5}$) of 3-*endo*-dimethylaminobornan-2-one methobromide is a better inhibitor than is the (−)-isomer (Ki $2{\cdot}5 \times 10^{-3}$), (Beckett & Griffiths, unpublished), see Fig. 3; the zone of steric hindrance interferes with the substrate receptor affinity in the (−)-isomer but not in the (+)-isomer.

(*ii*) In some aminoketones derived from (+)-bornan-2-one:

(a) When the nitrogen head is attached directly to the bornane ring (i.e. 3-*endo*-dimethylaminobornan-2-one methobromide) the compound possessing the trimethylammonium group produces a better inhibition (Ki $6{\cdot}7 \times 10^{-5}$) than does its corresponding protonated tertiary amine (Ki $1{\cdot}62 \times 10^{-4}$).

(b) The introduction of a methylene group between the bornane ring system and the head increases slightly the inhibitory effect of the protonated tertiary amine (Ki $1{\cdot}15 \times 10^{-4}$) but reduces that of the corresponding trimethylammonium compound (Ki $6{\cdot}18 \times 10^{-4}$).

(c) The introduction of a second methylene group between the bornane ring system and the head further increases the inhibitory effect of the protonated tertiary amine (Ki $2{\cdot}98 \times 10^{-5}$) but reduces further that of the trimethylammonium compound (Ki $2{\cdot}5 \times 10^{-3}$), (Beckett & Griffiths, unpublished).

(a), (b) and (c) can be explained as follows:—

In (a), the trimethylammonium head can bind to the anionic sites with its three methyl groups at E_1, E_2 and E_3 and at the same time, the carbonyl group can bind to the acidic function of the esteratic site. The corresponding protonated tertiary amine can bind to the active site in a similar manner except that it can only bind to the anionic

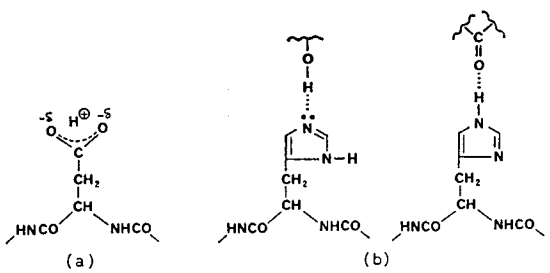

FIG. 2 (a). The ionized carboxyl of the aspartyl residue as the E_1, E_2 anionic receptor areas. (b) H-bonding of OH and $\diagdown C{=}O$ to the different tautomers of the imidazyl group of the histidyl residue.

site through two methyl groups and one proton; thus its lesser inhibitory activity is explicable.

In (b), some flexibility results from the introduction of the methylene group; the trimethylammonium compound cannot interact with both the anionic sites and the esteratic site without one of the methyl groups of the onium head interacting with the bornane ring system. In the corresponding protonated tertiary amine, this disadvantageously placed methyl group is replaced by the smaller hydrogen atom at E_3 so that steric interaction with the ring does not occur when the molecule is appropriately located at the receptor.

In (c), the trimethylammonium head and the carbonyl group cannot fit simultaneously at the anionic sites and the esteratic site without causing strong interaction between the onium methyl group at E_3 and the ring system; its corresponding protonated tertiary amine can bind at both sites without producing these steric interactions.

In some 2,3-substituted bicyclo (2,2,2) octanes, the difference in inhibitory activity between the *cis*-quaternary alcohol (Ki $6{\cdot}0 \times 10^{-5}$) and its corresponding protonated tertiary amine (Ki $3{\cdot}23 \times 10^{-4}$), and between the *trans*-quaternary alcohol (Ki $4{\cdot}335 \times 10^{-4}$) and its corresponding protonated tertiary amine (Ki $1{\cdot}89 \times 10^{-3}$) and also between the activities of the *cis*-quaternary acetate (Ki $4{\cdot}61 \times 10^{-4}$) and its corresponding protonated tertiary amine (Ki $1{\cdot}41 \times 10^{-4}$) and between the *trans*-quaternary acetate (Ki $9{\cdot}6 \times 10^{-4}$) and its corresponding protonated tertiary amine (Ki $2{\cdot}59 \times 10^{-3}$) may be explained similarly.

(*iii*) The introduction of the methyl group on the carbon atom bearing the ether oxygen of acetylcholine (i.e. β-to the N atom) reduced the rate of hydrolysis (Beckett, Harper & Clitherow, 1963); the L-(+)-isomer was hydrolysed 54% that of the parent whereas the D-(−)-isomer was not hydrolysed and had weak inhibitory activity. On the other hand, methyl substitution at the α-position to the N atom produced slight reduction in the rate of hydrolysis i.e. D-(+)-isomer; 78% and the L-(−)-isomer 97% hydrolysed. These results are explicable in terms of the receptor shown on Fig. 1b as follows:—

When the onium head of the D-(−)-β-isomer is appropriately located at the anionic sites,the interaction of the ester function with the esteratic site is almost precluded because its correct location would result in steric interaction between the E_3 methyl group and the β-methyl group. Thus binding of the isomer via its quaternary head can occur, but the ester function cannot be correctly located for hydrolysis.

For the L-(+)-β-isomer, when the head is correctly located, the ester function can interact with the esteratic site in a manner similar to that of acetylcholine but the β-methyl group will cause some rotation between the C_4–C_5 bond with consequent reduction in interaction between the ether oxygen and the acidic function of the esteratic site.

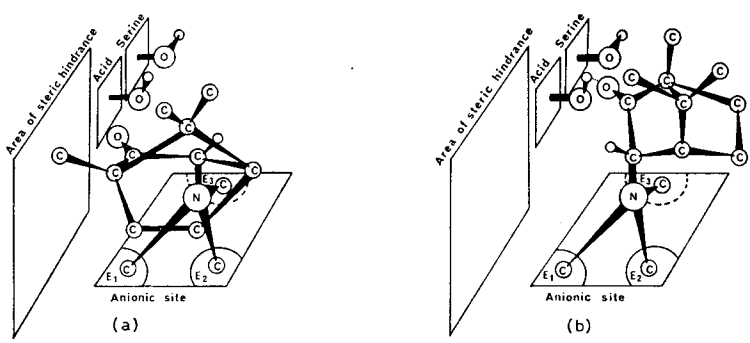

FIG. 3. The association of the enantiomers of dimethylaminobornan-2-one methobromide with the proposed new acetylcholinesterase receptor site. (a) (−)-isomer. (b) (+)-isomer.

The D-(+)-α-isomer can assume a conformation at the active site similar to that of acetylcholine, but the α-methyl group will be directed towards the area of the steric hindrance thus altering the ideal substrate conformation and slightly reducing the binding to the esteratic site. On the other hand, the L-(−)-α-isomer can fit both the anionic sites and the esteractic site as well as can acetylcholine because the α-methyl group is not now exerting any steric interaction with either the area of steric hindrance or the esteratic site.

(*iv*) In the 2,3-disubstituted bicyclo (2,2,2) octane series, the *cis*-quaternary alcohol (Ki $6{\cdot}0 \times 10^{-5}$), and its corresponding protonated tertiary amine (Ki $3{\cdot}23 \times 10^{-4}$), are better inhibitors than their corresponding *trans*-isomers (Ki $4{\cdot}335 \times 10^{-4}$ and $1{\cdot}89 \times 10^{-3}$ respectively). The *cis*-ester methobromide (Ki $4{\cdot}61 \times 10^{-4}$) and its corresponding protonated tertiary amine (Ki $1{\cdot}41 \times 10^{-3}$) are slightly better inhibitors than their corresponding *trans*-isomers (Ki $9{\cdot}6 \times 10^{-4}$ and $2{\cdot}59 \times 10^{-3}$ respectively).

This can be explained by the *cis*-compounds binding to the active site shown in Fig. 1b easier than do the *trans* isomers because, in the latter, some strain of the ring system is required for a hydroxyl group (or an ether oxygen of an acetate) to interact with the esteratic site when the onium head is appropriately located at the anionic sites.

(*v*) In the 2,3-substituted bicyclo (2,2,2) octanes, the alcohols were better inhibitors than were their corresponding esters, i.e. Ki values of the *cis*-quaternary alcohol ($6{\cdot}0 \times 10^{-5}$) and for its corresponding protonated tertiary amine ($3{\cdot}23 \times 10^{-4}$) and for their corresponding esters $4{\cdot}61 \times 10^{-4}$ and $1{\cdot}41 \times 10^{-3}$ respectively. The Ki values for the *trans*-quaternary alcohol ($4{\cdot}335 \times 10^{-4}$) and for its corresponding protonated tertiary amine ($1{\cdot}89 \times 10^{-3}$) and for their corresponding esters are $9{\cdot}6 \times 10^{-4}$ and $2{\cdot}59 \times 10^{-3}$ respectively.

These results can be explained as follows:—

The alcohols can bind to the acidic function of the esteratic site via the oxygen atom of the hydroxyl group and also by the onium head at the anionic site. In the case of the acetates, two factors are considered to lower the inhibitory activity:—

(a) The electron withdrawing carbonyl group reduces the electron density on the ether oxygen of the ester and thus reduces the strength of the binding of the latter to the esteratic site.

(b) The ester group has a greater sterically disadvantageous interaction with the receptor (active site) than has the smaller OH group.

One of us (A.A.B.) thanks the University of Riyad, Riyad, Saudi Arabia for a scholarship.

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June 10, 1975

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