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PATTERN RECOGNITION APPLICATIONS IN CHEMISTRY AND PHARMACOLOGY:--ETC(U)
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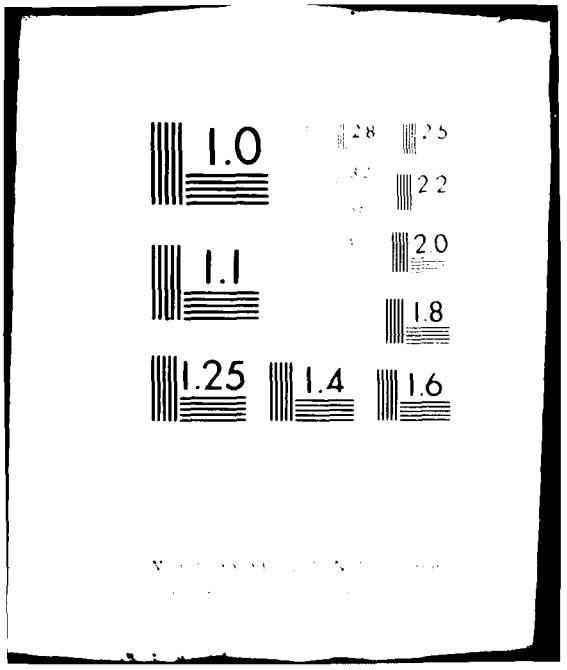
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PATTERN RECOGNITION APPLICATIONS IN CHEMISTRY AND PHARMACOLOGY: A 'PHARMACOPHORE ACETYLCHOLINORECEPTOR' SUBUNIT ENVIRONMENT MODEL

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In the analysis of a compound's structure for the prediction of its resultant pharmacological activity, the ultimate goal of the researcher is the explanation of the physiological mechanisms and compound interactions involved from the moment the drug is administered until the time it completes its effect. Having this knowledge, the researcher can then design compounds producing the efficaciousness desired. With this objective, investigators at the Research Division, Chemical Systems Laboratory, APG, MD have conducted chemical structure-biological activity relationship (SAR) studies (1) on various classes of compounds whose pharmacological actions 'in vivo' are directly related to the cholinergic system. The studies have included toxicity, cholinolytic and cholinomimetic activity, and the medical prophylactic and/or therapeutic efficacy of compounds or mixtures of compounds against anticholinesterase poisoning.

Of the possible receptors in this system, three types have been described (2) that are specific for interaction with acetylcholine (ACh). These are the nicotinic acetylcholine receptor (NAChR), the muscarinic acetylcholine receptor (MAChR), and the acetylcholinesterase enzyme (AChE). In this report, the general term 'acetylcholinoreceptor' (AChRE) refers to all three. Extensive research has been conducted on the pharmacological receptors and the biological mechanisms of action associated with the neurotransmitter acetylcholine (3-5) and many receptor configuration and biochemical mechanistic models have been proposed and modified as experimentation

has added new information (6-23). However, the precise environment of each acetylcholine receptor (AChR) type has not been defined.

This report defines a generalized pharmacophore acetylcholinoreceptor environment (AChRE) model. It is a 3-dimensional description of a composite acetylcholine receptor and of the relative geometric positions of specific compound functional group-receptor interaction regions. The relationship of this 'generalized AChRE' model to the AChE-acetylcholine receptor-ionophore complex and its potential application in the above research as a compound-receptor interaction reference template are discussed.

METHODOLOGY.

A. Chemical Structures.

A search was made for studies of ligand binding on muscarinic receptor, nicotinic receptor, and acetylcholinesterase and for relevant physiological studies. Structures of the compounds were obtained from the articles (6-30) or from various drug description indexes (31-33). Structures of chemical compounds from our SAR studies were also used. The 3-dimensional configuration of each compound was constructed using Dreiding Stereomodels. If X-ray diffraction analyses or crystal structure data were available, this information (14, 24-30) was employed to define the most likely configuration; if not, basic stereochemical principles were followed in construction of the molecules. Table 1 gives examples of the types of compounds found and used in the model formulation. The compounds are of both rigid and flexible structure types.

B. Compound-Cholinergic Activity 'Feature' Selection.

The identification of the compound features to be evaluated involved consultation with experts, literature searches and analyses of both drug-cholinergic receptor interaction models of the receptor types (NAChR, MAChR, AChE) being investigated, and data from 'in vitro' and 'in vivo' biochemical and physiological research related to the cholinergic system. The features included 3-dimensional geometric interatomic distances, specific compound functional groups, physicochemical and electronic properties.

Pattern recognition and clustering techniques are used to identify, rank, and quantify these features as to their relevance to cholinergic activity and to proposed compound-receptor interaction models. Of the 3-dimensional conformational receptor (AChR and AChE) models analyzed for significant features, the concepts and models of

Sommer (18), Golikov (8), Khromov-Borisov and Michelson (10), Kabachnick (16), and Pauling and Petcher (14) have the qualitatively 'best fit' compound-receptor feature interface.

C. Design of the 'Acetylcholinoreceptor' Model.

The 3-dimensional receptor model of Pauling and Petcher (14) was used as an elementary template amenable to modification and incorporation of features based upon requirements evolving from our SAR studies.

The overall development and refinement of our model took place in three phases:

1. (a) The 3-dimensional structures of the rigid neuromuscular blocking agents that Pauling and Petcher studied, were built using Dreiding Stereochemical models. (b) Using these compounds, their drug-receptor interaction template model was reconstructed to a Dreiding model scale for a definition of the relative positions of each proposed binding area and location of lipophilic, hydrophilic, or other potential compound-receptor interaction regions.

2. Using the Dreiding models, each compound noted in section A was constructed and superimposed on the basic template, with specific atoms and physicochemical binding regions of the compounds oriented for a 'best' possible fit. Common 3-dimensional structural features of these compounds and their potential receptor binding regions were defined.

3. Features identified in section B were incorporated and the results were then applied to modify the basic template. In this manner specific chemical compound bonding areas were eliminated and other interaction regions and physicochemical and macromolecular features were added until the proposed 'pharmacophore acetylcholinoreceptor' model was developed.

RESULTS. - 'Pharmacophore Acetylcholinoreceptor' Environment Model.

A. 3-Dimensional Geometric and Structural Components.

Figures 1 and 2 show the general AChRE model and its important interaction regions as conceptualized within a folded and partially closed or restricted area of a continuous membrane, the lower and upper surfaces of which are designated as A and B, respectively, and whose inner surfaces are at most 4.8 Å apart. Specific reaction sites and locations on the receptor are suggested

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by the nature of the chemical, electronic, or physicochemical binding potentials and characteristics present in the analyzed compounds. It is important to note that the model does not necessarily represent a single receptor that might actually be found in vivo, but is a composite of the several receptor models previously noted and the modifications and inclusions resulting from our structural studies. The membrane surfaces serve as a reference framework to assist in visualizing and defining the placement, extent, and limitations of various compound interaction regions. For clarity, the convention used to define the electronic (cation, anion) interaction sites of the receptor should be noted. In this report, these sites are defined in reference to the electronic charge of the compound interacting at these areas. By definition, the cationic site of the receptor refers to a negative area (labeled ⊖ in the figures) to which a postively charged region (cation) of a compound may interact. The anionic site of the receptor refers to a positive area (labeled ⊕) to which a negatively charged (anion) region of a compound may interact.

The basic structural features (Figures 1,2,3,4,5) of the model are:

1. A hydrophobic or van der Waals interaction ridge shown as a 3-dimensional cloud between and surrounding the cationic sites. Eight methylene groups can be accommodated along this ridge between the cationic regions. This is in agreement with the Pauling and Petcher model (14).

2. Two cationic sites, designated 1 and 2, separated by a distance of 11 Å along the van der Waals ridge and located 1.3 Å above Surface A (Figure 4). These sites provide binding areas for a compound's positively charged atom or functional group.

3. Three anionic sites, designated 1, 2, and 3, one of which is located on or slightly dimpled into Surface A, while the others are located on Surface B (Figure 5), optimally, at 2.2 Å (Site 2) and 2.3 Å (Site 3) above Surface A. These are sites of binding for an oxygen atom or oxygen isosteres.

4. Two planar lipophilic (hydrophobic) or π-π charge transfer binding regions, one of which is located on Surface A and is about the size of the planar tricyclic anthracene ring system. The second area of this type is smaller, about the size of a benzene ring, and is located on the inner side of Surface B, optimally at 2.6 Å above the plane of Surface A. These sites provide areas for planar group interaction with the receptor.

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5. Methyl or methylene group affinity regions are defined around both cationic sites (Figure 3).

6. Conformational allosteric flexibility of the receptor template, i.e., the proposed receptor can change its conformation causing displacement of its bonding areas and thus is capable of undergoing "allosteric" changes.

Figure 3 is a view from above the receptor model. This view necessarily superimposes portions of some features but shows the interaction regions in their optimal relative positions for acetylcholine interaction. All referenced distances are in angstrom units (Å). In this view the receptor is divided into 4 quadrants differentiated by X and Y axes which are orthogonal. The geometric positions can be calculated from Figure 3 and specific chemical atom and structural types which may preferentially interact with the general regions are referenced.

Figures 4 and 5 show the receptor features associated with Surfaces A and B. The cationic sites, van der Waals ridge, and methyl or methylene group affinity regions are considered as common to both surfaces. The 3-dimensional perspectives of Figures 1 and 2 illustrate this concept. Cationic site 2 is located in an environment that is more lipophilic or hydrophobic than cationic site 1. The anionic site 1 located on Surface A prefers hydroxyl -OH type interaction. Anionic site 2 on Surface B prefers esteratic oxygen -O- or sulfur -S- type interaction while anionic site 3 prefers carboxyl, phosphoryl, or hydroxyl type bonds. Also on Surface A, there is an additional methyl type interaction region (C-9) located adjacent to the 8 carbon methylene bridge separating the two cationic areas. In combination with methylene binding sites C-5 and C-6 on the van der Waals ridge, this site forms a hydrophobic pocket for a compound (Neostigmine) so structured as to require an affinity for this region.

Surface B can undergo greater allosteric changes than Surface A. These changes result in alterations of the positions of the methylene and oxygen binding regions (anionic sites 2 and 3). Surface B is loosely bound to Surface A so that it can easily be opened along the hydrophobic ridge and 'flap' like a page in a book (Figures 1 and 2). The degree to which it can undergo conformational change is limited. The maximum possible separation of the inner faces of Surfaces A and B appears to be 4.8 Å (14) based on compounds thus far studied.

B. Compound-Receptor Model Interactions.

In nerve or neuro-muscular cholinergic synaptic systems, the actual 'in vivo' position of true acetylcholine receptors in relation to the post-synaptic membrane and the synaptic cleft has not been definitively established. Our model can be positioned to encompass a receptor located on a membrane surface, embedded in a membrane, or combinations of these two, with the possibility of partial extension of the receptor from the membrane into the synaptic cleft. In Figure 2, all dimensions and positions of the interaction regions are referenced to the lower plane (Surface A). In this model, compounds interacting with Surface A are not hindered by possible 'receptor' structural components located on the Surface A parallel to the van der Waals ridge and where there is no overlap by Surface B. The limitationson a compound binding to Surface A are due to the compound's inherent lipophilic or π-π characteristics and to its degree of geometric planarity.

It is proposed that compounds interacting with the receptor approach the cleft formed by Surface A and the van der Waals ridge area. In order to attach to Surface B, a compound must bind to the receptor either along the ridge and to binding areas adjacent to the ridge, or in some manner create an opening between the two surfaces, thereby allowing the molecule to attach to the π-π regions and/or the anionic binding regions of Surface B that are otherwise masked by the unknown membrane structural components above and to the right of the inner face of surface B (see Figures 1 and 2). As is indicated in the figures, and symbolized by the slit in the right end membrane enclosing the subunit model, it may be possible to enlarge this area.

C. Compound-Receptor Stoichiometry.

The stoichiometry of a compound interacting with the receptor depends on its geometrical size, the positions of its potential interaction groups relative to each other and to the receptor binding regions, and its degree of lipophilicity or hydrophilicity. Accordingly, the model is a 3-dimensional template or puzzle diagram into which the 3-dimensional structural components of the compound are to be fitted. One or two molecules of a compound may bind to this pharmacophore model, or a compound may not bind at all. This pharmacophore receptor model can accommodate two acetylcholine molecules. As shown in Figure 6, the molecules are bound to the common cationic areas, to the hydrophobic ridge, and to corresponding anionic regions of Surface B.

*ASHMAN, THORNTON, BROOME, KING, & SACCO

DISCUSSION AND RECOMMENDATIONS.

A. SAR Studies.

The proposed composite model contains parameters identified 'qualitatively' as similar to those suggested for the various types of acetylcholine receptors (NAChR, MAChR, AChE); it should not be construed as one specific receptor type. The important aspect of this model is that it can be used in biochemical ligand binding studies and SAR cholinergic activity studies as a basic 'pharmacophore' template for compounds or mixtures of compounds interacting with the cholinergic system, both for better definition of the individual receptor environments and for improvement of the design of drugs having a desired pharmacological or physiological effect. The model can be used to locate compound substituent groups in relation to potential interaction areas on the receptor template; and thereby, one can use more efficiently the SAR methods of Hansch (34) or others (35,36) in the analysis of congeneric series of compounds.

Activities that can be investigated include toxicity, anticholinergic activity, cholinomimetic activity, anticonvulsant activity, acetylcholinesterase inhibition, and reactivation of inhibited enzymes by oximes.

It is suggested that investigators reconstruct the proposed model (at least the 2-dimensional level of Figure 3) to the scale of stereomodels (Dreiding) and then use the model to position to scale stereomodels of the compounds of interest for their 'best fit' on it. It is recommended that the model be constructed in two sections, as defined previously, and the sections overlapped with the edge of Surface B positioned above Surface A along the axis formed by the van der Waals ridge and the two cationic areas. Surface A should be placed so that it is stationary and all its binding groups remain on its plane. Surface B should be flexible to allow for conformational change of the position of its binding groups due to interaction of the compounds on the receptor.

In SAR analyses, it is suggested that the following features be included:

1. Compound-Receptor Interface. - A compound must penetrate between Surfaces A and B in order to attach to the binding areas on Surface B. It cannot penetrate from the right of the hydrophobic ridge as viewed in Figures 1 and 2.

2. Stoichiometry. - Stoichiometry is important in studying the cholinomimetic activity of a compound. Various biochemical studies (9,12) indicate that two cholinergic binding sites may be involved in the mechanism of the resultant nervous transmission. It also has been proposed (12) that there are two sites on the AChR having different affinities for acetylcholine molecules. The hypothesis is that one molecule-site interaction modifies the receptor conformation to allow for interaction of the second molecule of acetylcholine at a second site, or in some manner, the first interaction modulates the activity. The resultant pharmacological activity of a compound, therefore, may be correlated with the number of molecules that can bind.

3. Position of the Compound on the Model.- It is suggested that the model be divided into 4 regions labeled J, K, L, M as in Figure 7. Compounds believed to bind to region J are suggested primarily as antagonists of cholinergic activity. In general, this receptor region may be considered a regulatory area of either the AChR or AChE.

Region K contains the hydroxyl (OH) oxygen binding area (anionic site 1); most compounds binding here are anticholinergics.

Regions L and M contain interaction areas related to those of compounds having agonistic cholinomimetic activity. Generally, compounds that can fit one molecule in these regions without having part of its structure extended into region K display cholinomimetic activity. Also, regions L and M can be related to the active site of acetylcholinesterase.

Examples of compounds positioned to the receptor and their pharmacological actions are as follows:

a. Benactyzine can be positioned in regions K, L, and M. Its activity is anticholinergic.

b. Diazepam fits in region J. It is an anticonvulsant.

c. Phencyclidine could fit all 4 regions. Two of its molecules can be positioned without sterically hindering each other; one molecule positioned in regions J and K and one in regions L and M. Depending on dosage, its action can be inhibition of AChE, or interaction on the AChR-ionophore complex (37).

d. The oxime, 2 Pam Chloride fits all 4 regions. The regions (L,M) are assumed to be similar to the active site on AChE

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and we believe these regions are oxime reactivation activity regions. The possibility also exists that regions (J,K) may be regulatory (22, 38) areas for actions occurring at regions (L,M). Certain oximes may also interact with the AChR.

e. Waksman (39) used a series of fluorescent acyl-cholines that displayed agonist and antagonist activity on an isolated nicotinic AChR. We propose that these compounds can be positioned on the model and their resultant fit could qualitatively define their activity. The agonist compounds fit the receptor in a position similar to that of one decamethonium molecule or 2 acetylcholine molecules.

4. Separation of Surfaces A and B. - It is suggested that a measurement be taken of the inner surface separation made when the compound is positioned between Surfaces A and B. The degree of separation may indicate antagonistic cholinergic action possibly by interfering with the ionophore mechanism (8,19). This mechanism occurs in all 4 regions.

B. Drug Design Studies.

The model can be used in drug design studies as follows:

1. The structural and physicochemical feature characterization of compounds for isolation of efficacious parameters related to cholinergic and AChE activity.

2. Recommendation of new compounds to test, incorporating structural and physicochemical features suggested by the model.

3. Indication of possible new directions (new lead series of compounds or new combinations of compounds to test) that may increase efficacy or improve a proposed model.

4. As an aid in the development of mechanisitc and structural models of the 'in vivo' AChR-AChE-ionophore complex.

An example of a drug design study where the AChRE model has effectively been used is in the development of improved medical treatment of organophosphate poisoning. The organophosphate compound Soman (Table 1) is an anticholinesterase agent. Bullock (40) has reported that Soman can attack not only AChE irreversibly, but at high concentrations may also bind to the nicotinic AChR. Our composite receptor model predicts this possibility and allows for

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analyses of interactions with both receptors. Also, two molecules of Soman can bind to the model. In essence, a compound acting not only at different regions of the individual cholinergic receptors, but also at more than one receptor-type poses a greater toxicity hazard. This situation would probably require a more intense medical Prophylactic and therapeutic regimen.

Classical treatment of organophosphate poisoning involves the use of an oxime for reactivation of the inhibited AChE and the use of atropine to counteract the muscarinic effects of excess acetylcholine. Kepner and Wolthuis (41) reported on the therapeutic efficaciousness of the oxime HI-6 and atropine against Soman posioning in mice and rats. Using this mixture, via intramuscular route of administration in mice, a protective ratio of 17 LD_{50} (42) against Soman poisoning was obtained. Our objective was to increase this Protective ratio by the inclusion of a third compound into the mixture. Using the model as a guide, compounds with the following characteristics were recommended for syntheses or to be obtained: (a) stoichiometry of two, (b) oxygen or its isostere positioned in relation to anionic sites 2 and 3, (c) nitrogen at cationic site 1, (d) planar π-π structure positioned as on Surface B; (e) rigid ring structure along hydrophobic ridge (separation of Surface A from Surface B).

The compound 2-ethylamino-2(2-thienyl)cyclohexanone has the recommended features and its functional groups can be positioned geometrically to fit the model. In combination with HI-6 and atropine, it had a protective ratio of 29 (42) against Soman poisoning when tested as above.

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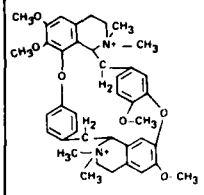
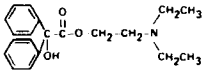
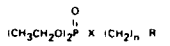
NICOTINIC RECEPTOR	MUSCARINIC RECEPTOR	ACETYLCHOLINESTERASE
		
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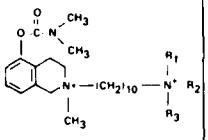	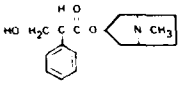	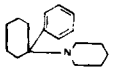
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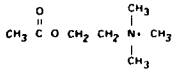	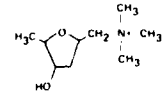	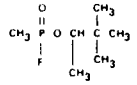
ACETYLCHOLINE	MUSCARINE	SOMAN

TABLE 1 EXAMPLES OF CHEMICAL COMPOUND CONFIGURATIONS

ACETYLCHOLINORECEPTOR MODEL

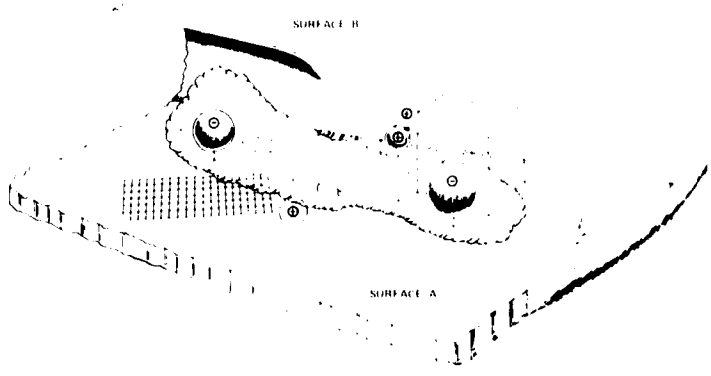

FIGURE 1 THE "PHARMACOPHORE ACETYLCHOLINORECEPTOR" SUBUNIT ENVIRONMENT MODEL SURFACES A AND B ARE CONTIGUOUS CROSSHATCHING IDENTIFIES AREAS OF POSSIBLE INTERACTION OF PLANAR AROMATIC MOIETIES WITH THE RECEPTOR THE ANIONIC AND CATIONIC AREAS ARE SHOWN AS SPHERES WITH THEIR CHARGE NATURE INDICATED THE CLOUD REPRESENTS A POTENTIAL VAN DER WAALS INTERACTION SPACE

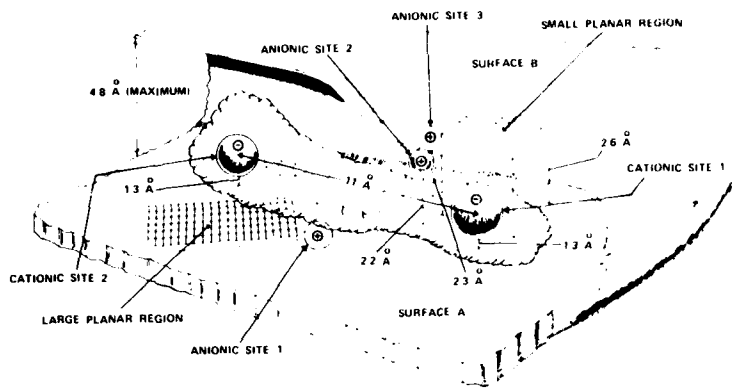

FIGURE 2 DIMENSIONS OF THE "PHARMACOPHORE ACETYLCHOLINORECEPTOR SUBUNIT ENVIRONMENT MODEL AS IN FIGURE 1 THE CLOUD REPRESENTS A POTENTIAL VAN DER WAALS INTERACTION SPACE THAT ENCOMPASSES BOTH CATIONIC SITES AND IS VERTICALLY BOUNDED BY SURFACES A AND B

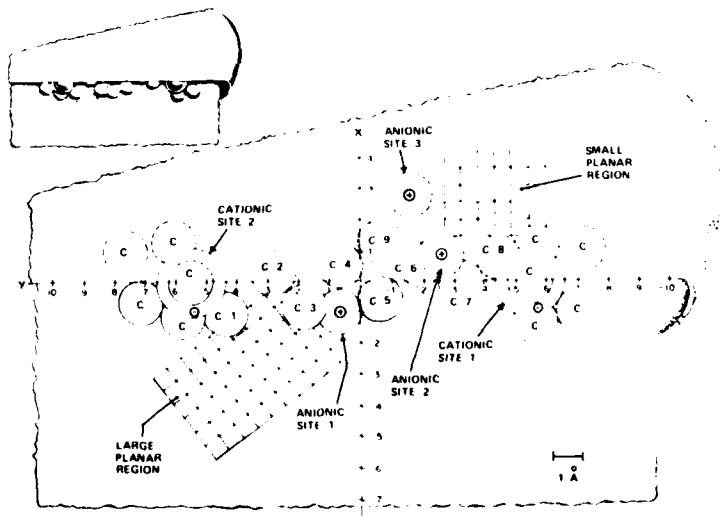

FIGURE 3 TOP VIEW OF THE RECEPTOR MODEL C_1 C_8 REPRESENT POTENTIAL METHYLENE CHAIN BINDING SITES EXTENDING ALONG THE VAN DER WAALS RIDGE BETWEEN THE CATIONIC SITES THE LATTER PROVIDE POSITIVELY CHARGED CATION BINDING AREAS C 9 AND THE UNSCRIPTED C_1 AROUND EACH CATIONIC SITE REPRESENT METHYL OR METHYLENE GROUP AFFINITY REGIONS CARBONYL ETHER OR HYDROXYL OXYGEN ATOMS AND ISOTERES OF OXYGEN S OR Se WOULD BE EXPECTED TO BIND AT THE ANIONIC SITES WHILE THE PLANAR REGIONS PROVIDE SITES FOR AROMATIC OR LIPOPHILIC INTERACTIONS

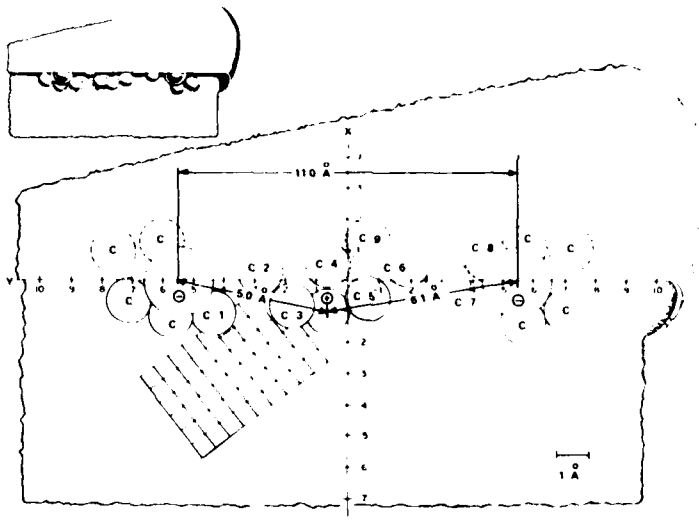

FIGURE 4 TOP VIEW OF THE RECEPTOR FEATURES ASSOCIATED WITH SURFACE A (SEE FIGURES 1 AND 2) THE CATIONIC AREAS, VAN DER WAALS RIDGE AND METHYLENE AFFINITY REGIONS ARE COMMON TO BOTH SURFACE A AND B AND ARE SHOWN FOR CLARITY AND ORIENTATION WITH REFERENCE TO FIGURE 3

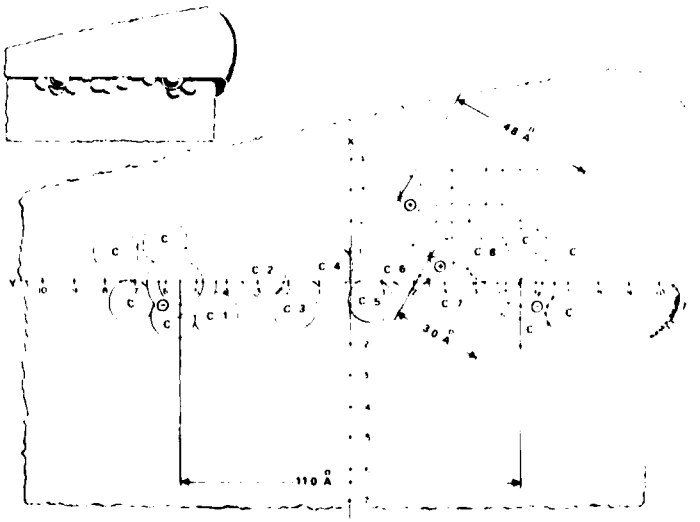

FIGURE 5 TOP VIEW OF THE RECEPTOR FEATURES ASSOCIATED WITH SURFACE B. THE CATIONIC AREAS VAN DER WAALS RIDGE AND METHYE OR METHYLENE AFFINITY REGIONS ARE COMMON TO BOTH SURFACE A AND B (REFER TO FIGURES 1 - 3)

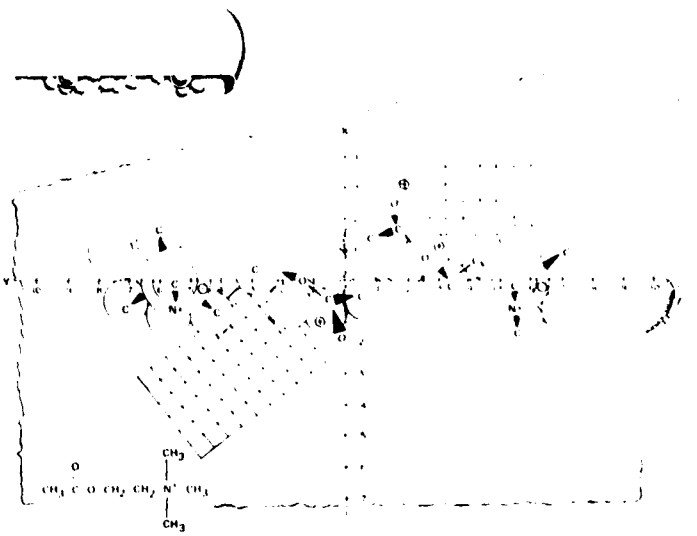

FIGURE 6 POSSIBLE ACETYLCHOLINE RECEPTOR INTERACTION WITH A STOICHIOMETRY OF 2

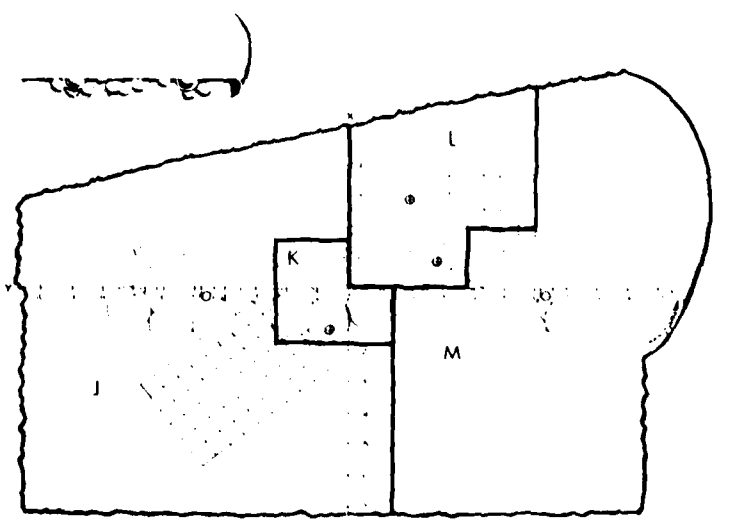

FIGURE 7 DIVISIONAL QUADRANTS OF THE PHARMACOPHORE MODEL

CHOLINORECEPTOR ANALYSIS QUADRANTS

J REGULATORY AREA (ANTAGONIST NON COMPETITIVE)
K AREA CONTAINING OH BINDING SITE
L *AGONIST BINDING AREA ESTERATIC AND ANIONIC REGIONS
M *AGONIST BINDING AREA (CATIONIC REGION

*DEPENDING ON STRUCTURE OF COMPOUND COMPOUNDS BINDING TO THESE AREAS MAY BE ANTAGONISTS