Structure Investigations of Agonists of the Natural Neurotransmitter Acetylcholine, V [1]

Structure-Activity Correlations for Cholinergic Stimulants Derived from Crystal Structures of Their Halides

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General features of crystal structures of halide salts of cholinergic stimulants can be interpreted in terms of substrate-receptor interactions. The monoatomic counterions in the crystal structures are discussed as models for the binding site of the receptor with respect to the ammonium group of the cholinergic neurotransmitters. In the crystal structures the anions occupy the tetrahedral faces of the quaternary trimethylammonio methyl or related groups in a specific geometry. Crystallographic and pharmacological evidence indicates that these groups should preferentially interact with the receptor via a specific face type (B-type face). The directionality of the interaction is derived from the vectors joining N^+ with the anions occupying B-type faces. So called "activity triangles", formed by the nitrogen of the ammonium group, a second polar centre of the neurotransmitter cation and a counterion occupying a B-face of the ammonium group, provide a structural criterion for the differentiation between muscarinic and nicotinic activity. It is shown that structure-activity relationships of cholinergic stimulants do not depend on the conformational details of the neurotransmitter cations, but primarily on the relative positions of the polar centres of the cations with respect to the anionic binding site of the receptor.

Introduction

A series of X-ray analyses of the agonists **1**–**8** (Fig. 1) of the natural neurotransmitter acetylcholine was carried out with the aim to obtain a better insight into the relationships between the structure and pharmacological actions of cholinergic compounds [2]. Our results take also into account the X-ray studies of cholinergic agents reported by other authors. The details of our X-ray structure investigations [2] are partially reported elsewhere [1, 3–5] and will be published further in the near future. In the present paper the discussion will be focussed on the correlations between structure and activity [2, 6, 7] which we derived from the crystallographic studies mentioned above. For the syntheses, the chemical and pharmacological properties of compounds **1**–**3** [8, 9], **4** [10], **5** [11], **6** [12, 13], **7** [14] and **8** [15, 16] we refer to the cited papers. **1**–**8**, contain a trimethylammonium group ($(H_3C)_3N^+-$), which is generally associated with optimum pharmacological activity [18]. A gradual substitution of the CH_3 groups of $(H_3C)_3N^+-$ by other alkyl residues or hydrogen normally reduces the activity successively [18, 19]. With the exception of **3**, compounds **1**–**8** contain at least one additional polar centre with a partial charge *i.e.* a carbonyl or an OH group, a nitrogen in a pyridine ring or an ether or ester oxygen. It is widely assumed that in addition to the ammonium group, at least one further (partially charged) group is involved in the biological activity [20–30].

1 exhibits a strong nicotinic activity, whereas the muscarinic one is very weak [9, 31]. This agrees with the experimental observation that the absence of an ether or ester oxygen in the same atomic position as in acetylcholine reduces muscarinic activity drastically, but nicotinic activity is nearly retained [32]. In contrast, the exchange of the carbonyl group of acetylcholine by a methylene group in **2** mainly affects nicotinic activity although muscarinic activity is also reduced [9, 31]. Generally chemical modifications of the ammonium group of cholinergic agonists affect muscarinic and nicotinic activity in an analogous fashion, whereas modifications of the carbonyl or the ester (ether) oxygen of acetylcholine and its

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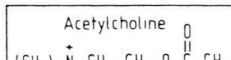
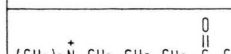
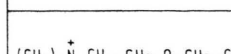
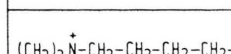
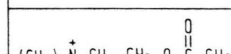
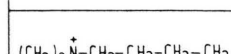
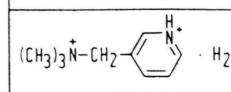
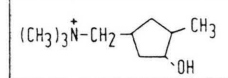
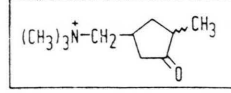
			Activity
	Acetylcholine 	X^-	M,N
1		Cl^-	N,wM
2		Cl^-	M,wN
3		Cl^-	N_G
4		I^-	N
5		I^-	N_G
6		$2Cl^-$	N
7		I^-	M,wN
8		I^-	M,N

Fig. 1. Chemical constitution, configuration and activity of compounds **1**–**8**. Code for activity: M = muscarinic, N = nicotinic, N_G = nicotinic on the ganglion, w = weak.

agonists have different influences on the muscarinic and nicotinic activity mode [32]. Compound **3** contains neither an ester (ether) nor a carbonyl oxygen. Both, muscarinic and nicotinic (at skeletal muscles) activity are remarkably reduced. However its nicotinic activity at ganglia exceeds that of acetylcholine [9, 31, 33, 34]. This is one of many indications suggesting the existence of two modifications of the nicotinic receptor, *i.e.* that of the skeletal muscles and that of the ganglion [34]. **5** exhibits an analogous cholinergic activity to **3** [11, 34, 35]. This compound contains an acetyl group which is differently positioned compared to acetylcholine. In both **5** and **3** the atomic positions which correspond to the carboxylate group in acetylcholine are exchanged by a CH_2–CH_2 moiety. **4** and **6** are nicotinic agonists. **4** contains the carboxylate group in the same position as acetylcholine, but the substitution of the acetyl residue by the next higher homologous propionyl residue results in a dramatic reduction of the muscarinic activity. It is known that on passing from acetylcholine to propionylcholine (**4**) and higher homologs, nicotinic activity increases reaching a test object dependent maximum in propionylcholine or butyrylcholine and decreases again in further higher homologs [22, 32, 36]. The nicotine derivative **6** is a potent agent exhibiting almost exclusively nicotinic activity [12, 31]. The pyridine nitrogen is in the same position as the carbonyl group of acetylcholine. Despite the absence of an ether oxygen, the muscarinic activity of **7** decreases only by a factor of 2–10 relative to muscarine. Its nicotinic activity is one order of magnitude lower than the muscarinic one [16]. The muscarone derivative **8** (like muscarone itself), exhibits significant muscarinic and nicotinic properties although, concerning muscarinic activity, the ether oxygen is absent [15, 16].

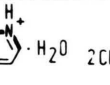
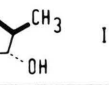
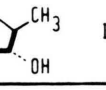
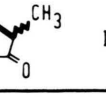
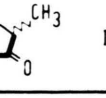
The problem of conformational flexibility in models for the structure-activity relationships

Several attempts have been undertaken to interpret the pharmacological behaviour of cholinergic compounds, *e.g.* the bimodal activity pattern of acetylcholine, in terms of their conformational flexibility [33, 37–40]. Within this framework, numerous spectroscopic investigations of cholinergic agents have been carried out [41]. In the crystal structures conformational flexibility manifests itself in form of statistically disordered structures, unusually high temperature parameters, or conformational variability for the same molecule either in different salts [42, 43] or even in the same crystal structure [44]. We also observed differing conformations in the crystal structure of **2** [4] and more or less extensive disorders in **2** [4], **3** [1], **4**, **7** and **8** [2, 17]. In the structures of **1** [3] and **5** [1] some high temperature parameters indicate a partial disorder.

This conformational flexibility complicates the elucidation of structure-activity relationships because crystalline structures are not necessarily pharmacologically active and extensive conformational changes may occur in the substrate-receptor interaction. Despite these ambiguities, the models which emerged in the past, were primarily based on conformations of cholinergic cations in crystalline state. Schueler [37] proposed for acetylcholine two active conformations, one of them relevant for the muscarinic, the other one for the nicotinic activity mode.

In contrast Chothia [45] assumed that the conformations relevant for the muscarinic and nicotinic activity mode of acetylcholine are very similar and proposed analogous active conformations for other cholinergic agents. The postulated active conformation [45–47] (*gauche-trans* referring to the torsion angles τ_2 and τ_3 in Table I) is exhibited in the crystal structures of acetylcholine chloride [48], in the perchlorate [49] and resorcylate [50]. Theoretical calculations have shown this conformation of acetylcholine to be the most stable one [51]. A different conformation *(gauche – gauche)* is found in acetylcholine bromide [52, 53], iodide [42], (±)-hydrogentartrate [43] and two modifications of the (+)-hydro-

Table I. Observed torsion angles τ_1–τ_4 for the cholinergic neurotransmitter cations of compounds **1**–**8**, compared with regions of torsion angles associated with muscarinic [47] and nicotinic [45] activity, as proposed by other authors. Code for activity: M = muscarinic, N = nicotinic, N_G = nicotinic on the ganglion, w = weak.

		τ_1	τ_2	τ_3	τ_4	activity
	muscarinic agonists (M) Ref. [47]	≈ 180	103 ± 34	180 ± 36	≈ 180 – 137	M
	nicotinic agonists (N) Ref. [45]	≈ 180	≈ 75	≈ 180	≈ 180 ≈ 0	N
1	$(CH_3)_3\overset{\tau_1}{\overset{+}{N}}-\overset{\tau_2}{CH_2}-\overset{\tau_3}{CH_2}-\overset{\tau_4}{CH_2}-\overset{O}{\overset{\|}{C}}-CH_3$ Cl^-	174	169	177	121	N,wM
2	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-O-CH_2-CH_3$ Cl^- a)	179	84	– 175	– 174 – 151	M,wN
	b)	– 162 – 171	81 69	169 – 179	101 – 179	
3	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-CH_2-CH_2-CH_3$ Cl^-	169	174	179	– 178	N_G
4	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_2-CH_3$ I^-	– 179	77	171	173	N
5	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_3$ I^-	178	– 171	176	65	N_G
6	$(CH_3)_3\overset{+}{N}-CH_2-$  $\cdot H_2O$ $2Cl^-$	171	90	177	3	N
7	$(CH_3)_3\overset{+}{N}-CH_2-$  I^-	167	155	155	– 168	M,wN
7	$(CH_3)_3\overset{+}{N}-CH_2-$  I^-	– 167	84	131	14	M,wN
8	$(CH_3)_3\overset{+}{N}-CH_2-$  I^-	cis isomer 171	163	163	– 164	M,N
		trans isomer 171	163	123	142	
8	$(CH_3)_3\overset{+}{N}-CH_2-$  I^-	cis isomer – 171	80	138	8	M,N
		trans isomer – 171	80	138	– 31	

gentartrate [43], and should therefore be regarded as irrelevant for cholinergic activity [45]. Based on this active conformation, Chothia [45] defined for the substrate a "methyl side" via which it binds with the muscarinic receptor and a "carbonyl side" for the binding with the nicotinic receptor. Furthermore, from the assumed pharmacologically active conformation, regions of torsion angles were deduced, which should characterize the conformations of cholinergic agents.

A first striking aspect of Chothia's approach is the fact that the postulated regions of torsion angles are very similar for the muscarinic and nicotinic mode of action [47, 45]. Furthermore, as it can be seen from Table I, some structures determined by us deviate significantly from the proposed conformations. In conjunction with this comparison, it must be taken into account that two of the conformational angles are normally fixed, *i.e.* τ_1 *(trans)* due to the staggered arrangement of the $(CH_3)_3N^+$ group relative to the N−C(−R)-bond, and due to electronic reasons τ_4 *(trans)* in the case of an ester group in the same position as in acetylcholine. Thus usually only τ_2 and τ_3 are variables. Deviations from the proposed conformational angles have also been reported [47] both for acetylcholine salts [42, 43, 53] and for a number of cholinergic agonists [54–58]. Therefore it can be generalized, that no unambigous correlation between the torsion angles obtained from crystal structures and biological activity can be established. Especially the torsion angles $\tau_1-\tau_4$ do not allow to distinguish between the nicotinic and muscarinic mode of action, a fact already pointed out by Chothia [45] and obviously demonstrated with compound **4**. The values of the torsion angles $\tau_1-\tau_4$ for this compound agree very well with the torsion angles often found in the case of muscarinic agonists. However as already mentioned, **4** exhibits an extremely low muscarinic activity, whereas its nicotinic activity is significantly higher than that of acetylcholine.

The anions in the crystal structures of cholinergic agonists as a model for the binding site of the receptor with respect to the ammonium group of the neurotransmitter cations

From the preceding discussion it becomes obvious that conformational angles found in crystal structures provide a more or less insufficient criterion for the differentiation between the muscarinic and nicotinic mode of action. For this reason we focussed our attention not only to the conformations of cations in the crystal structures, but also to the cation-anion interactions. It is widely accepted that the binding of cholinergic compounds with the receptor protein is mainly influenced by an ionic interaction, *i.e.* an interaction between a positively charged ammonium group and a complementary anionic group of the receptor [32, 59]. In addition, inspection of the crystal packing of compounds **1**–**8** reveals that it is mainly determined by Coulombic interactions between the cationic trimethylammonium group and the anions [2]. This type of interactions is believed to be similar to that occurring in the course of the transmitter-receptor binding. Thus the consideration of the packing features of cholinergic salts may provide a clue for a more realistic approach to the molecular basis of cholinergic actions. The present approach therefore treats the crystal model as a whole, including the anions.

If one considers in the crystal structures of **1**–**8** the arrangement of the monoatomic anions with respect to the quaternary ammonium groups in more detail, one finds that these are positioned specifically relative to this cationic group. The vectors between the anions in the first coordination sphere of the $(H_3C)_3N^+-CH_2-$ group and the nitrogen are directed nearly perpendicularly with respect to the tetrahedral faces of the $(CH_3)_3N^+-CH_2-$ moiety in their centres of mass (with the exception of one tetrahedral face which we discuss later), *i.e.* in the direction of the elongation of the N^+−C-bond to the methyl or the methylene group positioned opposite (Fig. 2). In the ideal case the angle δ_4 (for definition see Fig. 2) between the N^+-anion vector and the N^+-C vector which is directed to the opposite methyl or methylene group is 180°. Hence the angles $\delta_1-\delta_3$ with the three other N^+-C directions result in 70.5°. In a trimethylammonio methyl group $((CH_3)_3-N^+-CH_2-(CH_2))$ the four tetrahedral faces can be differentiated into three types (Fig. 3). Face type A is formed by three methyl groups, types B and C by two methyl groups and the methylene group of the aliphatic chain. Type B and C provide different environments to the anions; an anion brought near to face B "sees" three H atoms whereas in the case of face C it "sees" two hydrogen atoms of methyl groups and the methylenic carbon of the aliphatic chain in β-position with respect to N^+ − or the H atoms of this carbon atom, respectively −.

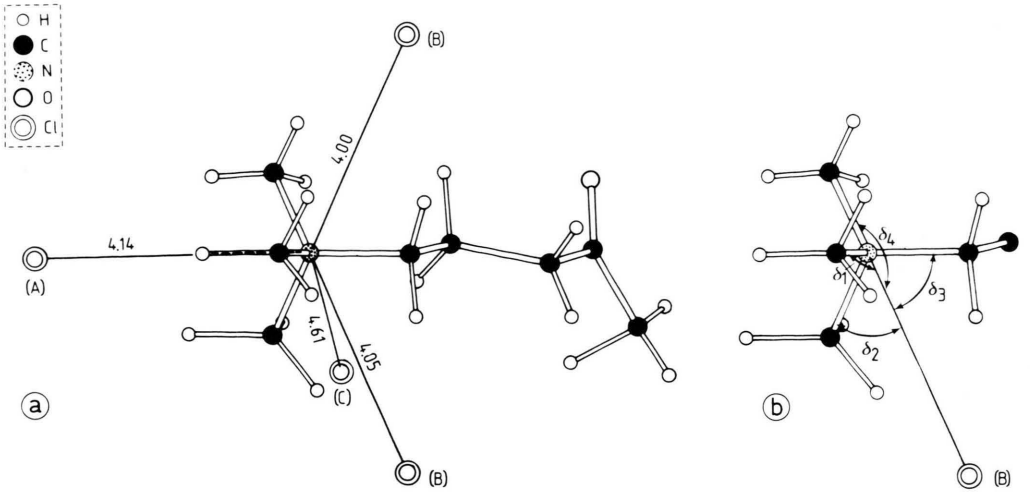


Fig. 2. a) Arrangement of the anions of the first coordination sphere relative to the quaternary ammonium group in the crystal structure of compound **1** [3]. The three distinct face types (defined in Fig. 3) of the tetrahedron formed around N^+ by the $(H_3C)_3N^+-CH_2-C$ group are indicated by A, B and C. Each tetrahedral face is "occupied" by one chloride anion. b) Definition of the angles $\delta_1-\delta_4$ for a B-type face.

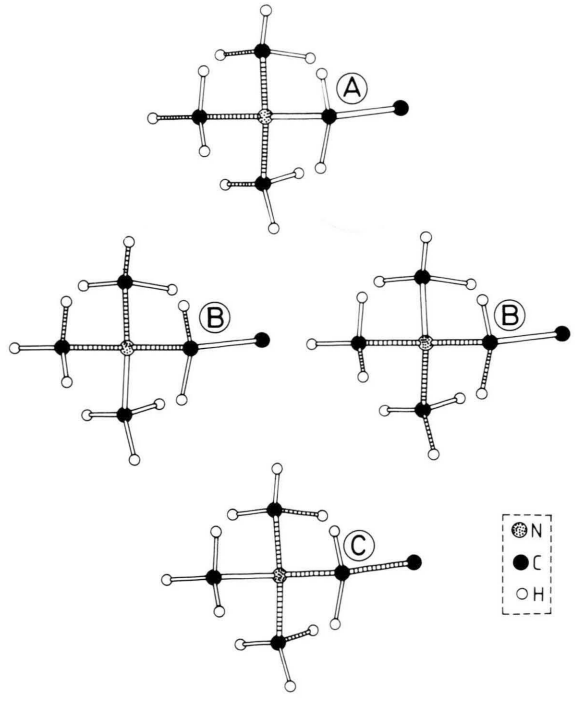
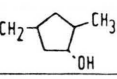
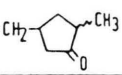
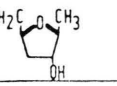


Fig. 3. Definition of the face types A, B and C of the tetrahedron at the nitrogen of the trimethylammonio alkyl group for compound **1** as an example. The letters are assigned in each case to the tetrahedral face which is put up by the three hatched N-C vectors.

Due to a local mirror symmetry, the face B always occurs twice. Therefore, the A face is defined by the *trans* (with respect to bond $N-C(H_2)$) and both the *gauche* methyl groups. Each of the two B faces is defined by the *trans*, a *gauche* methyl group and the CH_2 group and finally the face C is formed by the two *gauche* CH_3 groups and the CH_2 group.

In Table II the arrangement of the anions with respect to the tetrahedral faces of the trimethylammonio methyl group for compounds **1–8** and two additional structures taken from the literature are summarized in terms of the N^+-anion distances and the angle δ_4. Occupation of face C by anions is sterically unfavoured. The δ_4 angles (definition Fig. 2) show a wide variation and sometimes deviate significantly from the ideal value of 180°. Furthermore the N^+-anion distances are longer for face C compared with faces A and B. In each of the crystal structures mentioned, at least one B face is sterically optimally occupied, very often both B faces and the N^+-anion distances belong to the shortest found. In some cases the occupation of face A is also sterically favourable. In this case some exceptions can occur which in Table II are marked by hatched regions. In **6** the N^+-Cl^- distance is much too long and the angle δ_4 is unfavourable. A similar behaviour is observed in the structure of acetylcholine iodide [42], whereas in

Table II. Shortest N^+-anion distances (d) and the corresponding δ_4 angles, as defined in Fig. 2 for compounds **1**–**8** and two cholinergic structures, taken from the literature. Hatched areas are assigned to sterically unfavourably occupied faces of the tetrahedron of the trimethylammonio methyl group by anions.

		FACE TYPES					
		A		B		C	
		δ_4(°)	d (Å)	δ_4(°)	d Å	δ_4(°)	d Å
1	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-CH_3$ Cl^-	175	4.14	180 179	4.00 4.05	155	4.61
2	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-O-CH_2-CH_3$ Cl^- a)	172	4.13	175 165	4.15 4.34	155	4.72
	b)	174	4.16	171 173	4.18 4.22	155	4.72
3	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-CH_2-CH_2-CH_3$ Cl^-	159	4.17	179 170	4.10 4.32	170	4.32
4	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_2-CH_3$ I^-	176	4.39	175	4.52	163	4.72
5	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_3$ I^-	173	4.50	177 173	4.45 4.49	160	4.86
6	$(CH_3)_3\overset{+}{N}-CH_2-$  $\cdot H_2O$ $2Cl^-$	150	4.65	173 168	4.00 4.07	126 139	4.77 4.89
7	$(CH_3)_3\overset{+}{N}-CH_2-$  I^-	161	4.44	177 175	4.45 4.87	175	4.87
8	$(CH_3)_3\overset{+}{N}-CH_2-$  I^-	160	4.56	170 172	4.53 4.66	172	4.66
[42]	$(CH_3)_3\overset{+}{N}-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_3$ I^-	169	4.88* 4.92	175* 168/169	4.45 4.48*/ 4.52	160	4.71
[60]	$(CH_3)_3\overset{+}{N}-H_2C-$  I^-	—	—	165	4.49	157	4.79

* The two different values of this parameter arise due to a crystallographic disorder.

L(+)-muscarine iodide crystals [60] the A face is not occupied by anions. The specific orientation of the anions with respect to the N^+C_4 tetrahedron can also be found in other halides of cholinergic neurotransmitters with a $(CH_3)_3N^+-CH_2-R$ group. In Fig. 4 with respect to the anion occupation of faces B the δ_3 angles are plotted against the δ_2 for a series of crystal structures. For almost all of the δ_3/δ_2 pairs the deviations from the ideal values of δ_3 and δ_2 (70.5°) are smaller than 10°. If a few ghosts outside the hatched square in Fig. 4 are omitted, both δ_2 and δ_3 have an average value of 70.3°.

From the stereoselective arrangement of the anions with respect to the cations we have deduced that the anions in crystal structures of cholinergic stimulants can be used as a model for the main binding site of the receptor with respect to the neurotransmitter cations [2, 6, 7]. This assumption is supported by quantum mechanical calculations [68] which indicate that the above directionality is the preferred one for ionic interactions of the $(CH_3)_3N^+-CH_2-$ group. These theoretical studies come to the same result when the effects of solvent molecules are taken into account. This is particularly

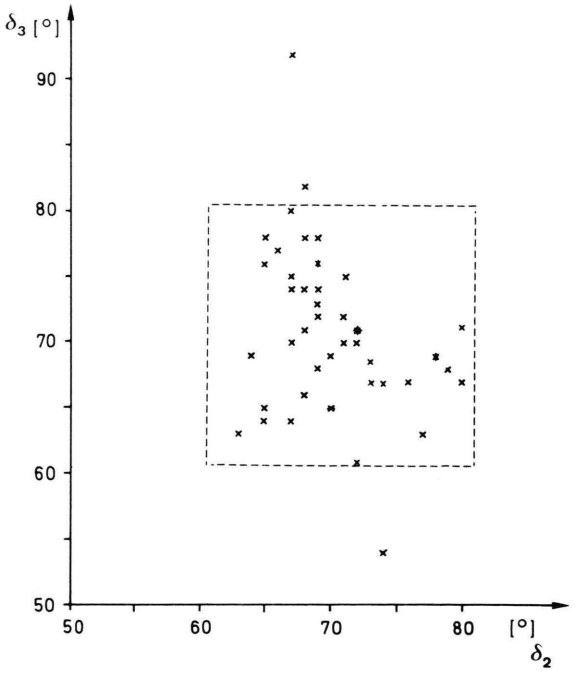


Fig. 4. δ_3/δ_2 diagram (for definition see Fig. 2) for B-type faces of the $(CH_3)_3N^+-CH_2-C$ group occupied by anions, in compounds **1**–**8** and halides of cholinergic stimulants, taken from the literature. Besides structures **1**–**8** the diagram contains values calculated from the coordinates of the following structures: acetylcholine iodide [42], chloride [48], bromide [53], L(+)-*cis*-2-(S)-methyl-4-(R)-trimethylammonium-methyl-1,3-dioxolan iodide [54], carbamoylcholine chloride [55], iodide [55], bromide [56], L(+)-muscarine iodide [60], (R,S)-lactoylcholine iodide [61], 2-trimethyl-ammoniummethyl-5-methyl furan iodide (5-methylfurmethide iodide) [62], L(+)-(S)-acetyl-β-methylcholine iodide [63], trimethyl-[4-(2-oxopyrrolidin-1-yl)but-2-ynyl]-ammonium iodide [64], γ-aminobutyric acid choline ester diiodide [65], 3-acetoxyprophyltrimethylammonium bromide [66], methyl 3-(dimethylamino)propionate methiodide [67].

important for our hypothesis, since it provides an approximation to the behaviour of the anionic group under physiological conditions. In addition our results [7] have been also confirmed by an extensive investigation [69] of the anionic environment in halides of the type $(CH_3)_3N^+-CH_2-R\ X^-$. Because the C faces are occupied by anions in a sterically unfavoured manner and there are some faces of type A with a sterically unfavoured occupation or no occupation, we have concluded that the cholinergic cations attack the receptor predominantly at faces of type B. Furthermore, in the cholinergic interaction face type B has an advantage relative to types A and C because normally B faces occur pairwise in the same geometry. The hypothesis of an adsorption of the cholinergic neurotransmitters at the receptor via one – or possibly both – B faces is supported by observations of the effects resulting from the substitution of methyl groups in the trimethylammonium group of acetylcholine by H or ethyl groups [18, 19]. It has been found that a drastic step in the loss of activity occurs when two or three CH_3 groups are replaced, whereas substitution of one CH_3 group has far less severe functional consequences. This behaviour fits to our hypothesis because (Fig. 3) substitution of only one CH_3 group leaves one B face unaltered, whereas further substitutions alter the original geometry of both B faces. In addition it is known that substitution of one CH_3 group by H in $(CH_3)_3N^+$ reduces the activity to a greater extent than a substitution by an ethyl group [19]. This can be explained in terms of our hypothesis because a B face with an ethyl group is capable of providing to an anion the same environment of H atoms as the one shown in Fig. 2, *i.e.* three H-atoms bound to C-atoms pointing in the direction of the anion; in contrast a H atom in place of the CH_3 alters this environment drastically. A further indication for the predominance of the B faces in cholinergic interactions is the fact that α-methyl derivatives of acetylcholine exhibit a reduced muscarinic activity [33]. This agrees with our model because substitution by methyl at the α-carbon of the chain R in $(H_3C)_3N^+R$ compounds blocks one B type face. On the other hand the A face remains sterically free, so that in view of the observed loss of activity, an interaction via this face type is at least unlikely.

To accommodate compounds which contain no $(H_3C)_3N^+-CH_2$ group (*e.g.* α-methyl substituted acetylcholine derivatives) in our model, we introduce the general definition of a B type face as the one formed by the α-carbon of the chain (α-position with respect to N), the substituent at the nitrogen in *trans* position with respect to this chain and either one of the two *gauche* bonding partners of the nitrogen. Otherwise, if no $(H_3C)_3N^+-CH_2-C$ group is present (*e.g.* nicotine [70]), the adsorption at the receptor should be achieved via a face which sterically fits best to a B face of the reference group.

We have also examined the arrangement of multiatomic oxyanions relative to the ammonium group. These anions should correspond to the real conditions at the receptor better than monoatomic anions,

because it is likely that the ammonium group interacts with receptor groups of type RCO_2^- [71]. In the crystalline environment however, these provide inconvenient model systems for in the case of sterically more extended anions other packing forces – including hydrogen bridges – can disturb the Coulombic interactions.

Differentiation of the mode of action on the basis of so-called activity triangles

The stereoselective arrangement of halide anions relative to the ammonium groups in crystals enabled us to postulate a model of the cation – anion interaction at the receptor. In the following a possibility to differentiate qualitatively between the modes of action by an extension of this model will be discussed. The agreement of this extension with experimental data supports indirectly the hypothesis, that the ionic binding of the substrate to the receptor occurs via faces of type B. Because a second polar group is normally necessary for the activity of cholinergic stimulants [20–30, 40, 51, 72], we have not only investigated the ammonium-anion vectors, but in addition the triangles formed by the quaternary nitrogen, an anion occupying a B face of the ammonium group and the second polar charge centre. In Fig. 5 two nearly mirror imaged triangles of this kind are shown which occur in compound **6**. Such "activity triangles" obey specific geometries depending on the mode of action. In Fig. 6 "activity triangles" for nicotinic agonists investigated by us are shown. These compounds exhibit a nicotinic activity at skeletal muscles. All triangles show an analogous geometry which is characteristic for this mode of action. Therefore we termed these triangles "activity triangles of the nicotinic mode of action". Compounds exhibiting a nicotinic activity only on the ganglion are omitted here. Activity triangles of several nicotinic agonists are superimposed in Fig. 7. This figure shows that independent of the details of their individual conformations, nicotinic agonists tend to form similar triangles. A relevant parameter for nicotinic activity is the angle between the vector connecting the polar groups in the cation and the N^+-anion vector. This angle (σ) varies in a relatively small range between 90° and 120°, with 100° on the average. Furthermore, the length of the vector between the polar groups in the cation is important. It is 5 Å on the average. A distance of this order (4.85 ± 0.1 Å) was already mentioned by Kier [74] as relevant for activity. This distance suggests that no triangles of this kind can be formed by an ester or ether oxygen in the position of the ester oxygen in acetylcholine, because the N^+-O distance is ca. 2 Å shorter. This result is consistent with the experimental observation [32] that an ether or ester function in the above mentioned atomic position is not relevant for nicotinic activity. We conclude from Fig. 7 that with respect to the mode of action of cholinergic compounds, the details of the conformation of the cations are not primarily relevant but rather the requirements imposed by the geometry of the activity triangles which have to be fulfilled by the conformation. Structures of muscarinic agonists form activity triangles in an analogous fashion to the nicotinic ones; the angle σ however is significantly smaller, *i.e.* in the region of 55°–80°. Characteristic muscarinic activity triangles (compounds **7** and **8**) are shown in Fig. 8, a superposition of such triangles is shown in Fig. 9. It reveals again that individual torsion angles are not relevant, but only the specific geometry of the vectors between the charged centres. It is also obvious that the anions attack the cations from a side which is free of sterical hindrance.

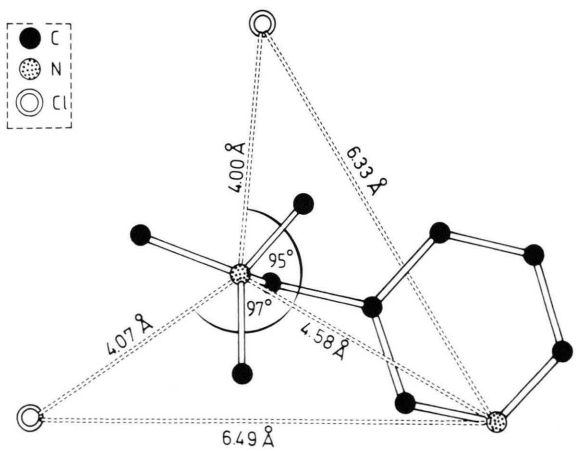


Fig. 5. Two nearly mirror imaged activity triangles in the crystal structure of **6**, characteristic for nicotinic activity at skeletal muscles.

Fig. 10 shows a convenient representation which allows one to distinguish between muscarinic and nicotinic activity on the basis of the activity triangles. In this diagram the apices of the activity triangles are orientated in one plane with coinciding N^+-anion

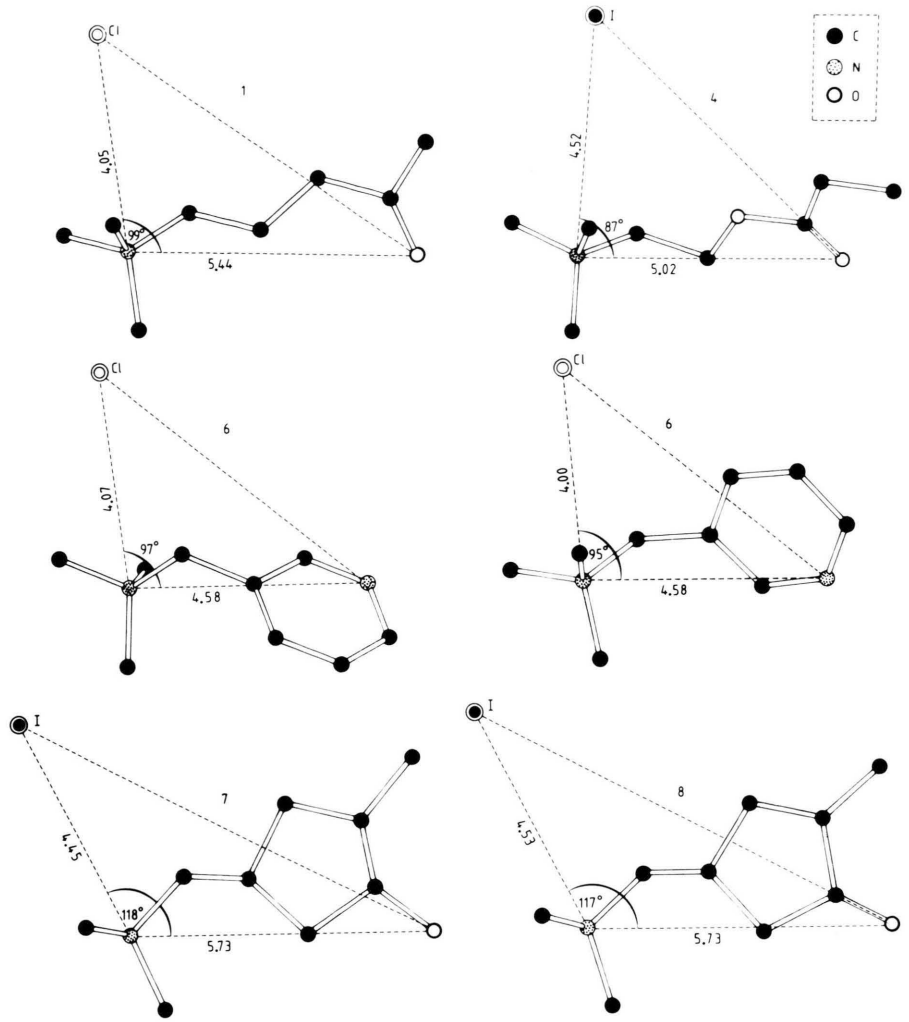


Fig. 6. Activity triangles for compounds exhibiting nicotinic activity at skeletal muscles, obtained from the crystal structures of **1**, **4**, **6**, **7** and **8**. The projection plane is defined in each case by the three apices of the triangle.

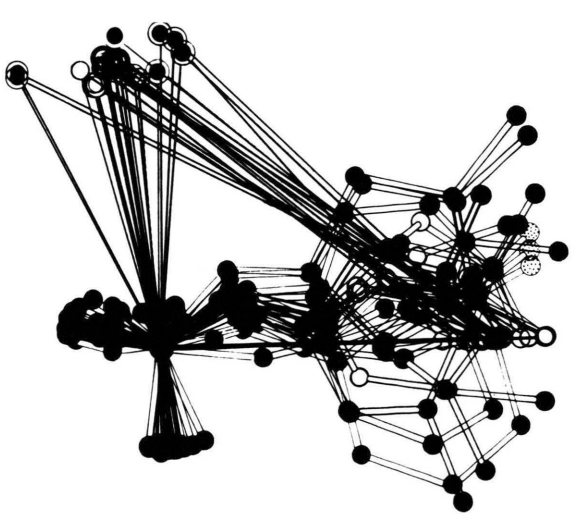


Fig. 7. Superposition of activity triangles of nicotinic agonists with activity at skeletal muscles. Projections are on the planes of the triangles. Besides the activity triangles shown in Fig. 6, nicotinic activity triangles found in the following crystal structures are incorporated: acetylcholine chloride [48], acetylcholine perchlorate [49], acetylcholine bromide [53], carbamoylcholine chloride [55], iodide [55], bromide [56], 3-acetoxypropyltrimethylammonium bromide [66], methyl 3-(dimethylamino)propionate methiodide [67], nicotine dihydroiodide [70], arecoline methiodide [73].

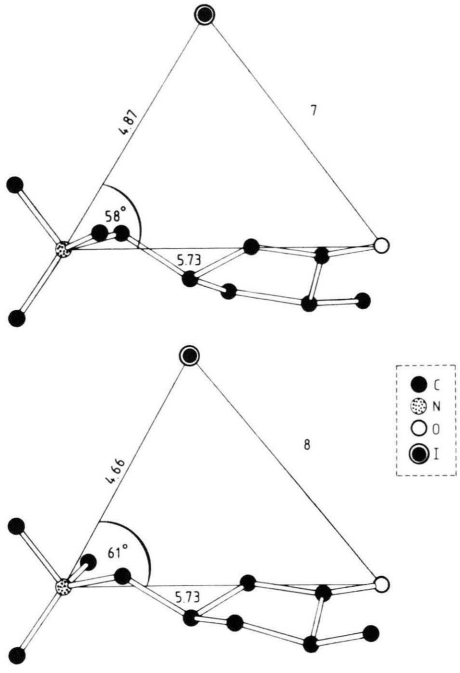


Fig. 8. Activity triangles in compounds **7** and **8**, which are characteristic for muscarinic activity.

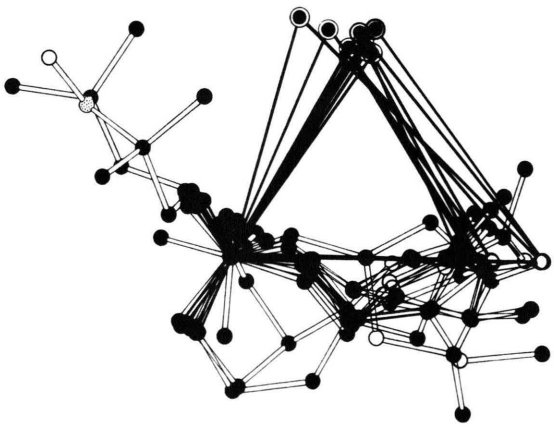


Fig. 9. Superposition of muscarinic activity triangles found in the following compounds: **7**, **8**, acetylcholine iodide [42], acetylcholine bromide [53], L(+)-*cis*-2-(S)-methyl-4-(R)-trimethylammonium-methyl-1,3-dioxolan iodide [54], "spin labeled" acetylcholine iodide [58], L(+)-muscarine iodide [60], 3-acetoxypropyltrimethylammonium bromide [66], arecoline methiodide [73], R(−)-3-acetoxyquinuclidine methiodide [75].

vectors. This forces a separation of the partially charged centers of the cholinergic cations into two different regions depending on their activity, *i.e.* region M for muscarinic and N for nicotinic activity. The agonists populating the region in between, *i.e.* the transition region T, exhibit in general a low muscarinic and/or a low nicotinic activity. As expected, muscarine is represented only in the muscarinic region and nicotine in the nicotinic one. Bimodal compounds *e.g.* the muscarone derivative **8** and acetylcholine itself, are represented in both regions. Compound **7** shows however that the differentiation given in Fig. 10 is primarily of qualitative and not of quantitative nature. **7** is found in the nicotinic as well as in the muscarinic region at almost the same positions as for compound **8**, although the nicotinic activity of **7** is very weak in comparison with that of **8** [16].

In the diagram of Fig. 10 we omitted compounds which exhibit exclusively or primarily nicotinic activity on the ganglion. If these compounds (*e.g.* **5**) contain a second polar group, the activity triangles formed (Fig. 11), differ from those (Fig. 7) formed by nicotinic compounds with activity at skeletal muscles. The angle σ remains the same, but the distance between the polar centers of the cation is elongated, in the case of **5** by about 2 Å. This agrees with literature data which indicate that nicotinic receptors occur in two modifications *i.e.* the receptor of skeletal muscles and the receptor of the ganglion [35]. However, as shown by the *n*-pentyl analog **3** of acetylcholine [1], a second polar group in the cation is not necessary for an activity at the ganglion.

There is also evidence for the existence of a second type of muscarinic activity triangles. Such triangles (not included in Fig. 10) are formed in **2** (Fig. 12) and exhibit as partially charged group an ether or ester oxygen in a position corresponding to the ester oxygen in acetylcholine. The distance between the N^+ and the second polar group is ca. 3.2 Å, *i.e.* approximately 2 Å shorter than in the case of muscarinic triangles of the first type. The geometry of this type of triangles, *i.e.* the N^+-O distance and the angle σ, depends only on the torsion angle τ_2, since τ_1 is fixed to ca. 180° due to sterical reasons.

The occurrence of this type of muscarinic activity triangles suggests that there are cations which can interact with different groups at the nicotinic and muscarinic receptors. For example in the crystal structures of the carbamoylcholine chloride [55], bromide [56] and iodide [55], the cation forms only

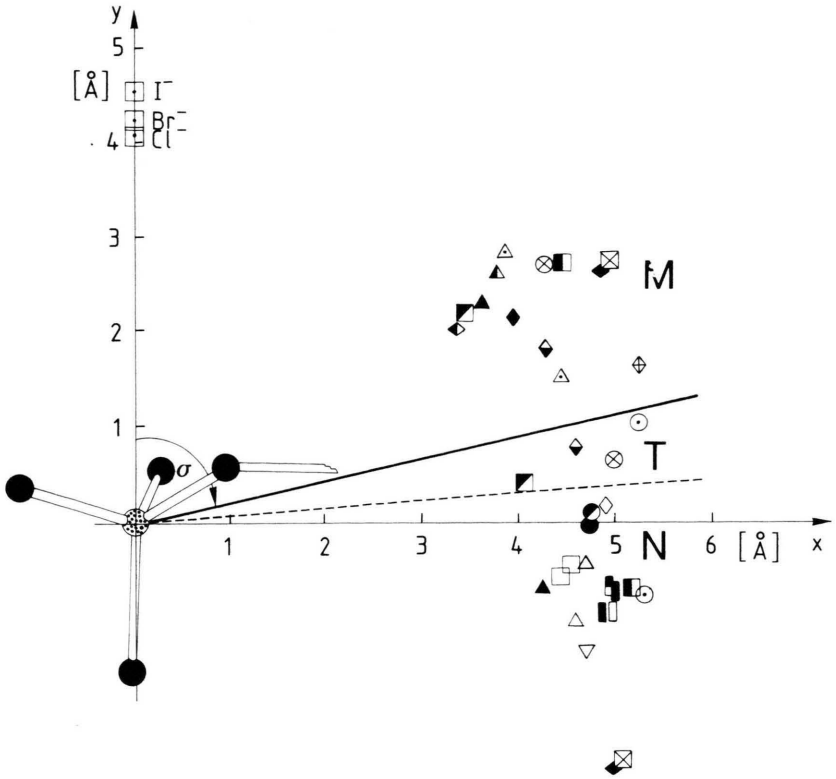


Fig. 10. Differentiation of nicotinic and muscarinic activity on the basis of nicotinic activity triangles (characteristic for the activity at skeletal muscles) and muscarinic activity triangles of first type (for second type see Fig. 12). The activity triangles lie in the *x*, *y* plane and are oriented in such a way, that the atom N^+ is placed on the origin and the vector N^+-counterion points into the direction of the *y*-axis. The vectors between the symbols of the compounds and the origin represent the vectors N^+-second polar center of the neurotransmitter cation. ⊙ = **1** [3], ◇ = **4** [17], □ = **6** [17], ◆ = **7** [17], ⊠ = **8** [17], ◭ = acetylcholine iodide [42], △ = acetylcholine chloride [48], ▽ = acetylcholine perchlorate [49], ▲ = acetylcholine bromide [53], ⬙ = L(+)-*cis*-2-(S)-methyl-4-(R)-trimethylammonium-methyl-1,3-dioxolan iodide [54], ▯ = carbamoylcholine chloride [55], ▮ = carbamoylcholine iodide [55], ▮ = carbamoylcholine bromide [56], ◬ = "spin labeled" acetylcholine iodide [58], ⊕ = L(+)-muscarine iodide [60], ◧ = 3-acetoxypropyltrimethylammonium bromide [66], ● = methyl 3-(dimethylamino)propionate methiodide [67], ◓ = nicotine dihydroiodide [70], ◩ = arecoline methiodide [73], ◆ = R(−)-3-acetoxyquinuclidine methiodide [75], ⊗ = (+)-(1S,2S)-*trans*-acetoxycyclopropyltrimethylammonium iodide [76], ◈ = 3(a)-dimethylamino-2(a)-acetoxy-*trans*-decalin methiodide [77].

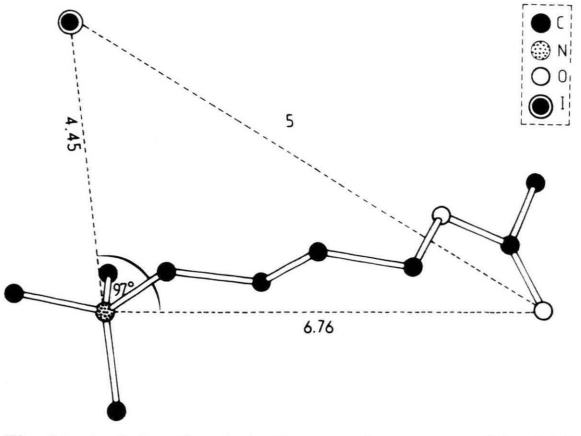


Fig. 11. Activity triangle in the crystal structure of **5**, which is characteristic for agonists with nicotinic activity on the ganglion.

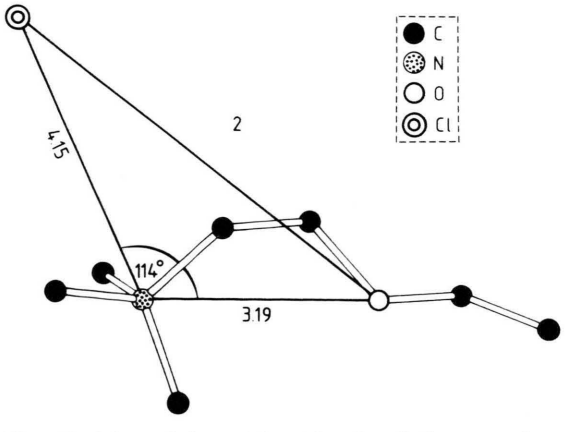


Fig. 12. Muscarinic activity triangle of the second type formed in the crystal structure of **2**.

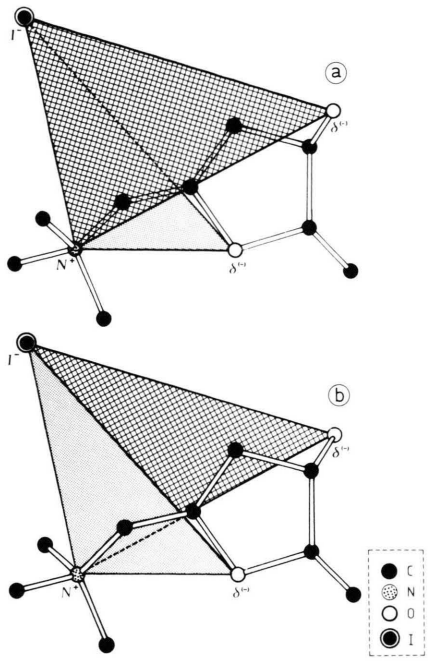


Fig. 13. a) Combination of muscarinic activity triangles of the first and second type for L-muscarine [60]. b) The mirror image of a) for D-muscarine.

nicotinic activity triangles with the carbonyl oxygen, whereas the ester oxygen gives rise to the formation of muscarinic activity triangles of the second type. Potent muscarinic agonists (*e.g.* L(+)-muscarine iodide [60]) normally form in their crystal structures both types of muscarinic activity triangles. Presumably these two muscarinic activity triangles have the N^+-anion vector in common. The occurrence of two types of muscarinic activity triangles indicates that two partially charged centres are favourable for adsorption at the muscarinic receptor. It may also help to explain the pronounced enantiomeric specificity of muscarinic agonists [78]. As in the case of L(+)-muscarine iodide [60], the two muscarinic activity triangles – of first and second type – form a chiral irregular tetrahedron (Fig. 13). If one takes the mirror image of muscarine this irregular tetrahedron is also mirror imaged. The "key" doesn't fit any longer into the "lock" of the receptor.

Activity triangles in salts of acetylcholine

In crystal structures of its halides, acetylcholine occurs in two different conformations depending on the counterion. With respect to the torsion angles τ_2 and τ_3 a *gauche-trans* conformation is present in the crystal structure of acetylcholine chloride [48], and a *gauche-gauche* in the crystal structures of the bromide [53] and iodide [42]. Due to their conformational differences, these forms of acetylcholine give rise to different combinations of activity triangles. In the *gauche-trans* form two nicotinic triangles and two muscarinic triangles of the second type are present. If one considers such combinations of activity triangles which have the N^+-Cl^- vector in common, one obtains two pairs of activity triangles, each pair consisting of one nicotinic and one muscarinic triangle of the second type. The *gauche-gauche* conformation allows in principle the formation of a nicotinic triangle, a muscarinic one of the first type and two muscarinic triangles of the second type. The combination of these triangles on the basis of common N^+-anion vectors yields the following pairs: A combination of one muscarinic triangle of first type with one of the second type and a combination of one nicotinic triangle with a muscarinic one of second type.

One can speculate that the differences in activity triangles between both conformations of acetylcholine may be correlated with the appearance of different activity modes. It is thus possible, that the strong muscarinic activity of acetylcholine is primarily associated with the *gauche-gauche* form which combines both types of muscarinic triangles in a similar fashion to potent muscarinic agents. On the other hand the *gauche-trans* form with two nicotinic triangles may be mainly responsible for the nicotinic response; an additional contribution from the *gauche-gauche* form which allows one nicotinic triangle is possible.

The contribution of the muscarinic triangles of second type to the total muscarinic activity of acetylcholine is uncertain. In general, the presence of only this type of triangles, gives rise to a muscarinic activity which is significantly lower than in the case of acetylcholine (*e.g.* compound **2**), although it appears possible that the muscarinic effects of this pair of triangles can be potentiated if they occur in the crystal structures with nearly ideal geometries, *i.e.* with the angle σ in the range of $\sigma = 90° \pm 10°$ (*e.g.* carbamoylcholine chloride [55], bromide [56] or iodide [55]). Thus it can be speculated that the conformation found in acetylcholine chloride in which the muscarinic activity triangles of second type only hardly fulfill the above geometrical requirements, will predominantly exhibit nicotinic actions. This

hypothesis is supported by compound **4** which has the same conformation as acetylcholine chloride *(gauche-trans)*, exhibits similar activity triangles and has almost exclusively nicotinic activity.

Hence we suggest that the *gauche-trans* conformation of acetylcholine is primarily involved in the nicotinic response, whereas the *gauche-gauche* conformation acts with one side as nicotinic and with the other side as muscarinic agonist. This proposal is an intermediate between the hypotheses of Chothia [45] and Schueler [37]: On one hand the same conformation is capable of producing both the nicotinic and muscarinic response, on the other hand different conformations are associated with different modes of action. Our model in addition restricts the degrees of freedom of the adsorption of the trimethylammonium group with respect to the counterions of the receptor. Whereas in Chothia's model [45] the orientation of the trimethylammonio methyl group with respect to the receptor is not restricted, in our model only a rotation around the N^+-anion vector, vertically to the B-face of the tetrahedron in the centre of mass of the face, is capable of establishing a fit of the second polar group. Finally it should be mentioned, that our approach can not be proved yet in detail and should be regarded as a hypothesis.

Acknowledgement

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