Recognition of Cholinergic Agonists by the Muscarinic Receptor. 1. Acetylcholine and Other Agonists with the NCCOCC Backbone

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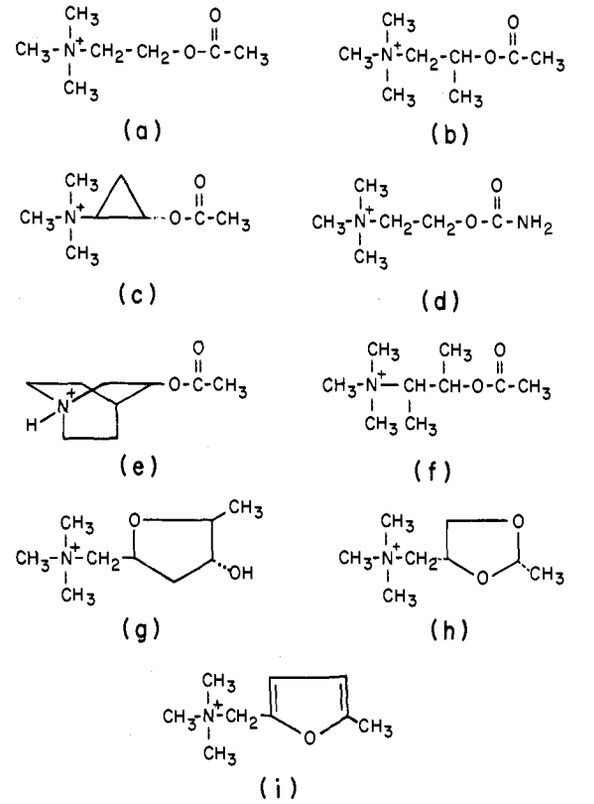
A theoretical model is used to deduce the pharmacologically active conformation of acetylcholine and other agonists interacting with the muscarinic receptor of the parasympathetic and central nervous systems. This is accomplished by replacing the usual dihedral angles τ_1 and τ_2, which define the conformations of cholinergic drugs, with two new geometric parameters more suitable for describing the muscarinic pharmacophore: a characteristic distance, $|PQ|$, and a dihedral angle, *PNOQ*. Values for these parameters are determined by conformational analysis on semirigid muscarinic agonists using molecular mechanics and ab initio molecular orbital methods. In addition to deducing the active conformation of acetylcholine and other agonists, the model also rationalizes the pattern of stereoselectivity in agonists related to 3-acetoxyquinuclidine (aceclidine) and furnishes a geometric criterion for partial agonism and antagonism.

A problem of great importance in neurochemistry is the manner in which a chemical neurotransmitter interacts with its corresponding receptor protein to initiate the series of steps that lead to a particular physiological response. The present paper contains a detailed analysis of the recognition of acetylcholine (Figure 1) and similar agonists containing the NCCOCC-type linkage (Chart I) by the muscarinic receptor of the parasympathetic and central nervous systems. It develops a theoretical model for the drug–receptor interacton deduced from a conformational analysis of semirigid muscarinic agonists; the requisite conformational energies are determined by a combination of molecular mechanics and ab initio quantum mechanical calculations. The model enables a determination of the muscarinic pharmacophore and, in particular, the biologically active conformation of each agonist. It rationalizes the unusual changes in potency associated with particular enantiomers of N-protonated and N-methylated 3-acetoxyquinuclidine (QNA), also known as aceclidine. The model suggests a size criterion for muscarinic drugs that correlates with their transitions from agonists to partial agonists. Finally, since the model identifies a specific conformation needed for activity, a straightforward test is the design of analogues that cannot attain this configuration by diverse, but minor, structural modifications and should therefore be inactive. Subsequent papers in this series will describe such analogues and will also apply the present model to muscarinics that do not contain the NCCOCC-type linkage, in particular, oxotremorine, muscarone, arecoline, and pilocarpine.

A. Aspects of the Structural Chemistry of Muscarinic Agonists. The subject of agonism and antagonism on the muscarinic receptor has been reviewed many times[1,2] and we give here only the salient structure–activity data relevant to the present model. It is well known that potent NCCOCC-type muscarinic agonists have a cationic head group (Chart I); a trimethylammonium group is present in all these agonists except N-protonated acetoxyquinuclidine (the neutral base is extensively protonated at physiological pH) and its N-methylated counterpart. For high potency, an ester or ether oxygen is required, since replacement of the oxygen with a methylene group[3] or sulfur[4] leads to diminished potency. Finally, a terminal alkyl group or equivalent, often in the form of a five-atom CCOCC chain, is required.[5] Decreasing or increasing the chain length by replacing the terminal methyl group of NCCOCC-containing agonists with hydrogens or larger alkyl chains results in a large decrease in drug potency. Sufficient lengthening of the alkyl chain leads to partial

Chart I. Structures of Nine Representative Agonists[a]



[a] a = acetylcholine; b = β-methylacetylcholine; c = ACTM; d = carbachol; e = protonated 3-acetoxyquinuclidine (QNAH); f = dimethylacetylcholine; g = muscarine; h = F2268; i = 5-methylfurmethide.

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(1) For some of the recent reviews of the muscarinic cholinergic system under consideration here, see (a) Cavallito, C. J. *Prog. Drug Res.* **1980**, *24*, 267–373. (b) Michel, R. H. "Cellular Receptors for Hormones and Neurotransmitters"; Schulster, D.; Levitzki, A., Eds.; Wiley: New York, 1980. (c) Sternlake, J. B. "Foundations of Molecular Pharmacology"; The Athlone Press: London, 1979; Vol. 2.

(2) For the present purposes we assume the existence of a prototype peripheral muscarinic receptor, largely independent of tissue and species in its recognition and activation characteristics. There is a growing literature on a multiplicity of muscarinic receptors of varying affinities in the nervous system; see Birdsall, N. J. M.; Hulme, E. C.; Burgen, A. S. V. *Proc. R. Soc. London, Ser. B* **1980**, *207*, 1–12.

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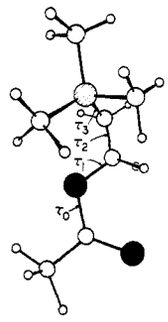

Figure 1. Acetylcholine (ACh) depicted with the nitrogen (cross hatched), the oxygens (darkened circles), and carbons (large, unfilled circles), and the hydrogens (small, unfilled circles). Four torsional angles are indicated with τ_2 (NCCO) and τ_1 (CCOC) being of principal interest.

agonism and ultimately to antagonism. Indeed, potent muscarinic antagonists, such as atropine, scopolamine, and quinuclidinyl benzilate, possess very bulky terminal ester groups. The chain length at which partial agonism first appears (which is dependent upon the tissue, perhaps via the number of spare receptors) differs for the various muscarinics in a way that will be discussed later.

Stereoisomerism in the muscarinic agonists also plays a significant role in drug potency; e.g., of the eight possible epimers of muscarine, only the naturally occurring L(+) form (2*S*,3*R*,5*S*) has appreciable pharmacological activity.[4a] Similarly, for ACTM the trans form (1*S*,2*S*) is the only potent isomer.[6]

While attempts to interpret the structure–activity data have met with partial success[7] no definite conclusions have been reached,[7f] and no testable model has been formulated. For example, from a number of X-ray diffraction studies of muscarinic agonists, Pauling and co-workers[8] have suggested a range of 70° for both conformational dihedral angles τ_2 (NCCO) and τ_1 (CCOC)[9] (Figure 1). In fact, the range of τ_2 is considerably larger, since the more active forms of QNAH and QNAMe are *S* and *R*, respectively, having dihedral angles of opposite sign. There is a limitation in the use of dihedral angles to describe the pharmacophore, namely, that they serve only to define the backbone of the drug and not the "interaction pharmacophore",[10] the collection of spatially arrayed molecular electronic attributes that characterize the interaction of the agonist with the receptor, such as the charge distribution, molecular electrostatic potential, etc. In the present model we define a new pair of geometric parameters (section B) that are more appropriate to the description of the muscarinic drug–receptor interaction.

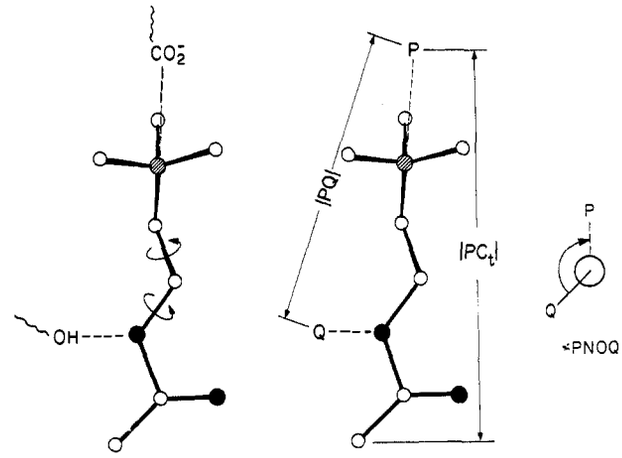

Figure 2. (a) Acetylcholine interacting with the receptor's carboxylate oxygen and an electrophilic group, such as a hydrogen-bonding proton. (b) The oxygen is indicated symbolically by *P* while the electrophilic site is located at the point of minimum electrostatic potential near the ester oxygen, denoted by *Q*. The interaction dihedral angle *PNOQ* is indicated on the right-hand side of the figure. Also shown are the distances |*PQ*| and |*PC*$_t$|.

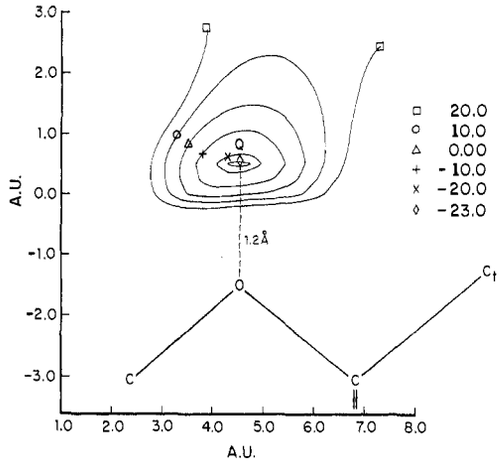

Figure 3. Contours of the molecular electrostatic potential of QNAH$^+$···OH$^-$ near the ester oxygen in the COC(=O) plane. The minimum of the electrostatic potential, *Q*, lies in the COC plane, 1.2 Å from the oxygen. The ab initio STO-3G basis set was employed.

B. A Model of Agonist Recognition by the Muscarinic Receptor. The following are the assumptions upon which the model is based and their rationale. (1) It is assumed that the cationic head group of the agonist interacts directly with an anionic receptor site, such as a carboxylate ion, and, furthermore, that there exists a very specific orientation of the agonist head group to the receptor oxygen. A reasonable possibility is that the receptor

(3) (a) Ing, H. R.; Kordilzad, P.; Tudor Williams, D. P. H. *Br. J. Pharmacol.* **1952**, *7*, 103–116. (b) Sundelin, K. G. R.; Wiley, R. A.; Givens, R. S.; Rademacher, D. R. *J. Med. Chem.* **1973**, *16*, 235–239. (c) Melchiorre, C.; Gualtieri, F.; Giannella, M.; Pigini, M.; Cingolani, M. L.,; Gamba, G.; Pigini, P.; Rossini, L. *Farmaco, Ed. Sci.* **1975**, *30*, 300–310. (d) Givens, R. S.; Rademacher, D. R. *J. Med. Chem.* **1974**, *17*, 457–459. (e) Melchiorre, C.; Angeli, P.; Giannella, M; Gualtieri, F.; Pigini, M.; Cingolani, M. L.; Gamba, G.; Leone, L.; Pigini, P.; Re, L. *Eur. J. Med. Chem.* **1978**, *13*, 357–361, and references therein.

(4) (a) Waser, P. G. *Pharmacol. Rev.* **1961**, *13*, 465–515. (b) Elfrenik, J. G. R.; Salemink, C. A. *Arzneim.-Forsch.* **1975**, *25*, 1702–1705.

(5) Ing, H. R. *Science* **1949**, *109*, 264–266.

(6) Chiou, C. Y.; Long, J. P.; Cannon, J. G.; Armstrong, P. D. *J. Pharmacol. Exp. Ther.* **1969**, *166*, 243–248.

(7) (a) Beckett, A. H.; Harper, N. J.; Clitherow, J. W. *J. Pharm. Pharmacol.* **1963**, *15*, (b) Bebbington, A.; Brimblecombe, R. W. *Adv. Drug Res.* **1965**, *2*, 143–172. (c) Kier, L. B. *J. Pharm. Sci.* **1970**, *59*, 112–114. (d) Beers, W. H.; Reich, E. *Nature (London)* **1970**, *228*, 917–922. (e) Chothia, C. *Ibid.* **1970**, *225*, 36–38. (f) Shefter, E. "Cholinergic Ligand Interactions"; Triggle, D. J.; Moran, J. F.; Barnard, E. D., Eds.; Academic Press: New York, 1971; pp 83–116.

(8) Baker, R. W.; Chothia, C. H.; Pauling, P.; Petcher, T. J. *Nature (London)* **1971**, *230*, 439–445.

(9) The dihedral angles τ_0 and τ_3 are always found in X-ray studies to be ca. 180°, corresponding to a staggered trimethylammonium arrangement and a planar ester group, respectively. We have found no reason to suggest otherwise.

(10) (a) Maayani, S.; Weinstein, H.; Cohen, S.; Sokolovsky, M. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 3103–3107. (b) Weinstein, H.; Maayani, S.; Srebrenic, S.; Cohen, S.; Sokolovsky, M. *Mol. Pharmacol.* **1973**, *9*, 820–834. (c) Weinstein, H.; Maayani, S.; Srebrenik, S.; Cohen, S.; Sokolovsky, M. *Ibid.* **1975**, *11*, 671–689.

oxygen lies on the threefold axis of the trimethylammonium group or is nearly colinear with the NH bond in the case of protonated head groups so as to provide a linear N–H···O hydrogen bond. Calculations showing that this is a favorable directionality for NO interaction have been performed by Weinstein et al.[10c] and our group for muscarinic drugs by using model anions such as OH^- and HCO_2^- by INDO semiempirical and ab initio methods, respectively. In order to proceed further, it is also necessary to assume a specific distance between the interacting nitrogen of the head group and the receptor oxygen. The calculations suggest that an NO distance of 3 Å is physically reasonable, and that value is adopted here. The left-hand side of Figure 2 depicts a carboxylate interacting with the ACh head group along the threefold axis; on the right-hand side of the figure, the oxygen is represented abstractly by a point P on the threefold axis 3 Å from N.

(2) It is assumed that a region of negative electrostatic potential proximal to the ester or ether oxygen corresponds to a second binding site. This region provides a stabilizing electrostatic interaction with a positive receptor residue or a hydrogen bond to a receptor hydrogen donor. Figure 3 shows the molecular electrostatic potential for $QNAH^+\cdots OH^-$ in the vicinity of the ester oxygen in the plane containing the COC(=O) group. The electrostatic potential minimum occurs at a point in the plane designated by Q in Figure 3, which is along the COC bisector and 1.2 Å behind the oxygen. The STO-3G (see section C) value of the energy of interaction of this potential with a protonic charge at Q is −23 kcal/mol. A point Q of similar potential and located by a similar vector OQ is found for all the NCCOCC agonists; it is depicted in the left-hand side of Figure 2 for the case of a hydrogen bond with the receptor, and abstractly by using the point Q on the right-hand side. In the case of interaction of the ester oxygen with some positive receptor entity, point Q would correspond to a position of positive charge on the receptor.

(3) Since P and Q are *receptor sites* and since agonist binding is of high affinity and stereoselectivity, it is reasonable to assume that the distance $|PQ|$ is a nearly invariant property of the receptor in its active conformation. A somewhat arbitrary but physically reasonable assumption in keeping with the high affinity and specificity is that $|PQ|$ varies by no more than 0.3 Å over the set of agonists.

(4) In order to define the relative orientation of the drug to the receptor, which should also remain nearly invariant over the set of agonists, we define the "interaction dihedral angle" between the two vectors NP and OQ (or the planes NPO and POQ) as the positive dihedral angle corresponding to the extent of clockwise rotation needed to superimpose OQ on a stationary NP, when viewed along the nonbonded O → N direction. This angle, denoted as $PNOQ$, is also indicated in Figure 2. It is reasonable to expect that only a narrow range of PNOQ values would characterize NCCOCC drug–receptor complexes. The new variables $PNOQ$ and $|PQ|$, which replace τ_1 and τ_2, put all the agonists on a common footing.

(5) Finally, in order to develop the model in detail it is necessary to evaluate the conformational energies of the known, potent muscarinic agonists and to determine the set of accessible PQ distances and $PNOQ$ angles that are common to all agonists. Therefore, an energy criterion is needed that distinguishes accessible from inaccessible conformations. The model assumes that a conformation is accessible if its energy is within 3–4 kcal/mol of the minimum-energy conformer. This amount of distortion energy is probably easily obtained from interaction of the drug with the receptor or bulk solvent, since it corresponds in amount to only an ethane torsional barrier. On the other hand, significantly larger distortion energies probably preclude binding to the receptor. The conclusions reached here, however, *do not* depend critically or solely upon the 3–4 kcal/mol criterion for accessibility, since the conformational energies are used in conjunction with other structure–activity data.

C. Methodology for Obtaining Conformational Energies. For ACh and other NCCOCC-type muscarinic agonists, the conformations are defined by τ_1 (CCOC) and τ_2 (NCCO). Their conformational energies can be displayed on a two-dimensional grid as functions of the dihedral angles so that the determinations of their conformational energies are two-parameter problems. This point must be qualified, however, since Gellin and Karplus have shown[11] that for the very hindered muscarinic agonist β-MeACh and presumably other hindered agonists, it is necessary to allow small adjustments ("relaxations") in the remaining 3N-8 internal coordinates at each τ_1, τ_2 point to preclude artificially high conformational energies.

Many of the semirigid agonists present one-parameter conformational problems. For example, the energies of ACTM and QNA can be analyzed as functions of τ_1 alone, since τ_2 is constrained by inclusion in rigid rings. Similarly, for muscarine, F2268, and 5-methylfurmethide, the conformational energies are functions of τ_1 alone. In one-parameter problems the remaining 3N-7 internal coordinates are relaxed.

The energies of the muscarinic agonists were obtained by quantum mechanical SCF calculatons at each conformation. However, although it is possible to calculate the ab initio energies of the agonists for a limited number of conformations, it is still too costly to determine the relaxed geometries, for which a much larger number of calculations would be required. We therefore adopted a hybrid approach in which the relaxed geometries are determined by the more rapid MM2 molecular mechanics method,[12] and the conformational energies at these geometries are then evaluated by ab initio calculations.

The MM2 conformational energies are obtained by summing empirical van der Waals, torsional, bending, stretching, stretch–bending, and dipole–dipole contributions. Since these energies can be computed rapidly at each geometry, optimization of the geometrical parameters by a Newton–Raphson method is rapid and inexpensive. A convenient feature of the MM2 program is that it allows geometry optimization with τ_1 and/or τ_2 constrained, which facilitates scans over the torsional angles. The MM2 geometry searches succeeded in reproducing the known X-ray geometries for the agonists studied.

The SCF calculations were implemented with the STO-3G contracted Gaussian basis set[13] and, at a few geometries of special interest, the larger and more accurate 4-31G basis set.[14] Good agreement between the results with these two basis sets was obtained, which is not surprising since both sets have been shown to furnish accurate torsional barriers.

It should be emphasized that the present calculations do not attempt to evaluate the binding energy between agonist and receptor but rather the effect of the drug–

(11) Gellin, B. R.; Karplus, M. *J. Am. Chem. Soc.* **1975**, *94*, 6996–7006.
(12) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134. Allinger, N. L.; Yuh, Y. H. *QCPE no. No. 395.*
(13) Hehre, W. G.; Stewart, R. F.; Pople, J. A. *J. Chem. Phys.* **1969**, *51*, 2657–2664.
(14) Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724–728.

Table I. |PQ| Values of Semirigid Muscarinic Agonists for Several *PNOQ* Angles

agonist	*PNOQ* angle:	|PQ| value, Å			
		70°	85°	100°	115°
muscarine		6.0	6.4	6.7	6.9
F2268		6.1	6.4	6.8	7.0
5-methylfurmethide		5.8	6.3	6.7	7.0
ACTM		6.6	6.6	6.7	7.0
(*R*)-QNAMe		6.1	6.1	6.3	6.5

receptor interaction on the accessible range of drug conformations. For one to obtain accurate binding energies, it would be necessary to know all the drug–receptor interactions, as well as the modification of the receptor conformational energy due to drug binding, information that will be unavailable for the foreseeable future.

In the present investigation it was necessary to calculate the molecular electrostatic potential (MEP), which is a measure of the tendency of a positive potential site to interact with the drug at various points in space. A useful application of the MEP to the serotonin recognition problem has been made by Weinstein et al.[15] As discussed in section B, the MEP serves to locate the point of minimum electrostatic potential proximal to the ester or ether oxygen. In this work the potentials were calculated in the STO-3G basis set.

Results

Determination of the Muscarinic Pharmacophore. By application of the present analysis to the semirigid agonists, it is found that the muscarinic pharmacophore corresponds to an angle *PNOQ* between 60 and 117°. The lower bound is provided by ACTM at a distortion energy of 4 kcal/mol; to attain a *PNOQ* value of 40° would require an unlikely 9 kcal/mol distortion energy. The upper bound, 117°, is provided by (*R*)-QNAMe; a 10 kcal/mol distortion energy is required for 122°. An important aspect of the accessible 60 to 117° range of *PNOQ* values is the fact that muscarine, F2268, and 5-methylfurmethide can attain *PNOQ* angles greater than 90° *only* if the ab initio energy calculations include a hydroxy group with oxygen at point *P* to model the interaction between the anionic receptor site and the cationic head group. The presence of the anionic group serves to dissipate an attractive ion–dipole interaction between the charged head group and the ester oxygen, thus allowing conformations with larger τ_2 and, in turn, larger *PNOQ* values, in addition to anchoring the drug to the receptor.

Having employed the constraint that the interaction dihedral angle be accessible to all muscarinic agonists, it is now useful to apply the constraint of a common *PQ* distance as well. Table I gives |*PQ*| values for ACTM, muscarine, F2268, 5-methylfurmethide, and (*R*)-QNAMe at *PNOQ* angles of 70, 85, 100, and 115°. At 70°, the agonist |*PQ*| values span the range 5.8 to 6.6 Å, and this is inconsistent with the ca. 0.3 Å variation in |*PQ*| postulated by the model.

The *PNOQ* values of 70 and 85° can also be ruled out for the muscarinic pharmacophore by a detailed study of (*S*)-β-MeACh.[16] The energy surface of this molecule (Figure 4) as a function of τ_1 and τ_2 (with relaxed geometries) was obtained by Gellin and Karplus[11] using a molecular mechanics method. We have checked their energies for a number of points on the surface using the MM2–ab initio procedure (STO-3G), including OH^-, to model the receptor anion and found agreement to within 1–2 kcal/mol.

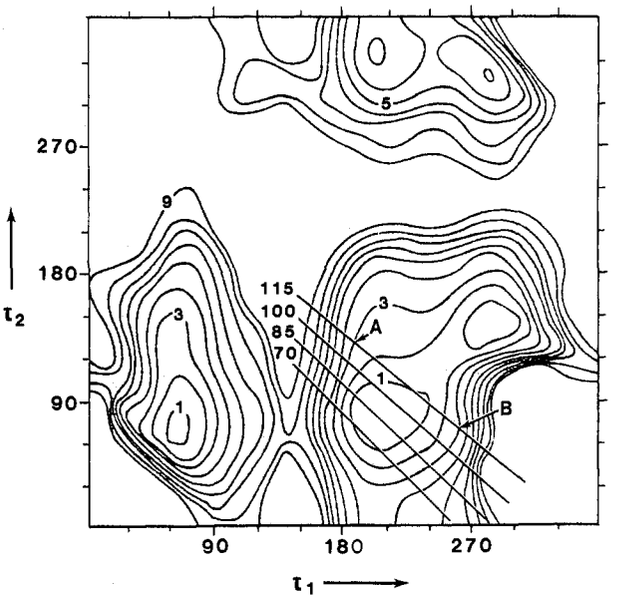

Figure 4. Conformational energy of β-MeACh.[11] Contour labels are in kilocalories/mole. Superimposed on the plot are lines of constant *PNOQ* for 70, 85, 110, and 115°. The points denoted by *A* and *B* represent the range of conformations consistent with the muscarinic pharmacophore. Point *A* corresponds to the active conformations of ACh and β-MeACh.

We have superimposed on the β-MeACh energy surface the loci of constant *PNOQ* for 70, 85, 100, and 115°, which, somewhat surprisingly, are a set of divergent straight lines. These lines are also, to good approximation, the loci of constant |*PQ*| for β-MeACh in the region of interest. For *PNOQ* = 70, 85, 100, and 115°, the β-MeACh |*PQ*| values are ca. 5.3, 5.5, 6.1, and 6.7 Å, respectively. From Table I it is seen that for ACTM the |*PQ*| values range from 6.6 to 7.0 Å. Thus, for |*PQ*| to be the same to within 0.3 Å for both β-MeACh and ACTM, *PNOQ* values of 70–100° must be rejected. The most satisfactory interpretation of the data, *taken as a whole* for all the agonists, is that the muscarinic pharmacophore corresponds to *PNOQ* values of between 100 and 117° and |*PQ*| varies from 6.6 to 6.8 Å. These are the drug-inferred pharmacophore values. At the higher *PNOQ* limit are β-MeACh and, by analogy, ACh; at the lower limit are muscarine, F2268, and 5-methylfurmethide. It might be noted that while the |*PQ*| value for (*R*)-QNAMe increases with its *PNOQ* angle, even at the largest energy-allowed value, 112°, it is still only 6.4 Å; this is 0.3–0.4 Å outside the above range and may be the source of the much lower potency of (*R*)-QNAMe compared to the other muscarinic agonists considered here.

Stereoselectivity of the Muscarinic Agonists. The present model of the muscarinic pharmacophore explains several aspects of agonist stereoselectivity. For one thing, enantiomers of the active forms of muscarine, F2268, ACTM, (*R*)-QNAMe, and β-MeACh cannot attain the muscarinic pharmacophore geometry at reasonable energetic cost, which is consistent with their inability to act as agonists.

An unusual case of agonist stereoselectivity occurs in the QNA series and is depicted in Figure 5. (*R*)-QNAMe has been found to be more potent than (*S*)-QNAMe on the guinea pig ileum, while in the protonated case, the order of potencies is reversed with (*S*)-QNAH more potent than

(15) Weinstein, H.; Osman, R.; Green, J. P.; Topiol, S. "Chemical Applications of Atomic and Molecular Electrostatic Potentials" Politzer, P.; Truhlar, D. G. Eds.; Plenum Press: New York, 1980.

(16) Ellenbroek, B. W. J.; van Rossum, J. M. *Arch. Int. Pharmacodyn.* **1960**, *125*, 216–220.

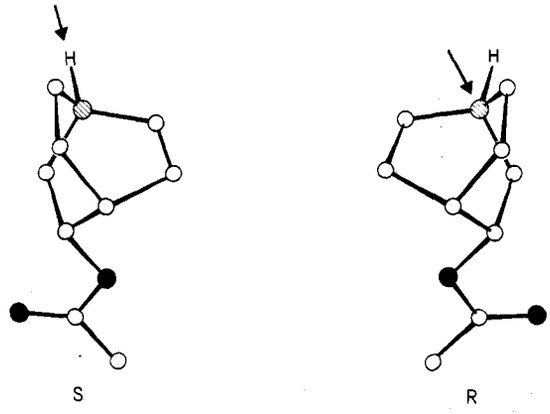

Figure 5. The *R* and *S* isomers of QNAH. Nitrogens are represented by cross-hatched circles, and oxygens are represented by solid circles. For (*S*)-QNAH, the approach most consistent with the pharmacophore is along the NH direction as shown (arrow). A much less energetically favorable approach is along a CN axis. The latter approach is used by (*S*)-QNAMe. For (*R*)-QNAH, the only direction of approach is that shown, which is used also by (*R*)-QNAMe.

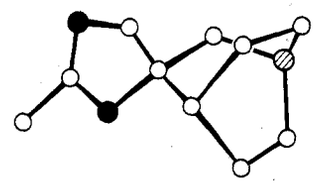

Figure 6. The weak, semirigid agonist MSDQ.

(*R*)-QNAH.[17] The ratios of the EPMR's are[17c] (*S*)-QNAH/(*R*)-QNAH/(*R*)-QNAMe/(*S*)-QNAMe = 5.7:34.5:146:>10^4. The relative potencies in the N-methylated case can be explained quite simply by the inability of (*S*)-QNAMe to attain the muscarinic pharmacophore configuration. The relative potencies in the protonated case can be understood if it is assumed that this system offers a new direction of approach (arrow) to the anionic receptor site, namely, along the NH direction, with the formation of a nearly linear N–H···O bond to the receptor oxygen. Investigation shows that this mode of approach is available only to (*S*)-QNAH, which can attain the muscarinic pharmacophore configuration with *PNOQ* = 112.5° and |*PQ*| = 6.6 Å for a distortion energy of 3 kcal/mol. It is not available to (*S*)-QNAMe, since the methyl group would be interposed between the head-group nitrogen and the receptor oxygen, nor is it available to (*R*)-QNAH, which would require considerable distortion energy to attain the pharmacophore configuration. Thus, (*R*)-QNAH utilizes the same mode of interaction with the receptor (arrow) as does (*R*)-QNAMe; not surprisingly, their potencies differ only by a factor of 4.2. As has been noted previously, this mode for (*R*)-QNA(H or Me) is associated with |*PQ*| values smaller than the range deduced for the muscarinic pharmacophore. Thus, the higher potency of (*S*)-QNAH is in accord with its better fit to the pharmacophore pattern and, therefore, a better interaction with the receptor. Barlow and Casy[17b] inferred that such a phenomenon occurs, but did not discuss its nature in detail.

An agonist related to QNA is protonated 2-methylspiro[1,3-dioxolane-4,3′-quinuclidine] (MSDQ), which is shown in Figure 6. The compound has been synthesized and shown to have a potency similar to QNAH by Fisher et al.[18] Their work involves an attempt to prepare a nearly completely rigid agonist by combining the τ_2 constraint of quinuclidine with the τ_1 constraint of dioxolane. Even so, the MM2–ab initio method shows that for 3 kcal/mol distortion energy, *PNOQ* can still vary from 30 to 115° [assuming that the active epimer corresponds to (*S*)-QNA] due to the flexibility of the dioxolane ring. At *PNOQ* = 114°, the |*PQ*| value is 6.6 Å; thus, MSDQ satisfies the requirements for a muscarinic pharmacophore as inferred in the preceding section.

Table II. Geometric Parameters for the Biologically Active Conformations of Muscarinic Drugs

agonist	*PNOQ*, deg	|*PQ*|, Å	|PC_t|, Å
ACh[a]	117.0 (115.7)	6.7 (6.5)	8.5 (6.2)
β-MeACh[a]	117.0 (116.3)	6.7 (6.5)	8.5 (6.5)
muscarine	104.9	6.8	8.9
F2268	100.6	6.8	8.9
5-methylfurmethide	100.6	6.8	8.5
ACTM	100.5	6.7	8.9
(*S*)-QNAH	112.5	6.6	7.5
MSDQ	114.1	6.8	7.8
(*R*)-QNAMe	111.7	6.4	8.1

[a] Values for conformation *A*, with conformation *B* values given in parentheses.

The Relationship between Chain Length and Potency. The relationship between the potency of muscarinic agonists and the nature and length of the terminal alkyl chain has been the subject of investigation for more than 30 years. In 1949, Ing[5] enunciated the "five-atom rule", which states that for a series of muscarinics of structure R-NMe_3^+, the most active agonist is that in which R contains a five-atom chain, excluding hydrogen. Several years later, the rule was amended to apply to a homologous series in which the terminal alkyl chain length varies, but the functional group—usually the ester or ether oxygen—remains in the same position relative to the head group.[19] Recent confirmation of these ideas is found in the work of Pratesi and collaborators[20] who have made QSAR studies of the affinities and potencies of muscarinics with various terminal chains based on benzyl-, picolyl-, and furfuryltrimethylammonium salts, alkyltrimethylammonium salts, and esters and ethers of choline. A typical example of the stringent chain-length requirements is found in the furmethide series, where 5-methylfurmethide is 35 times more potent than furmethide itself and 105 times more potent than 5-ethylfurmethide. (The latter compound is a partial agonist on some preparations.) High potencies are also observed for the "five-atom" agonists 5-chloro-, 5-bromo-, and 5-iodofurmethide. These results suggest that for high potency the distance between the anionic receptor site *P* and the terminal atom, usually a methyl carbon, should be similar to that of 5-methylfurmethide, which is 8.5 Å in the muscarinic pharmacophore. To illustrate this point, we have compiled these distances, denoted |PC_t|, for the other semirigid agonists in their active conformations in Table II. For ACTM, F2268, and muscarine |PC_t| is 8.9 Å. On the other hand, |PC_t| in

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furmethide is 7.1 Å, while in the "six-atom" system 5-ethylfurmethide it is 9.7 Å. Thus, when the terminal atom is farther from P than 9.7 Å, partial agonism or antagonism is observed, while $|PC_t|$ distances much less than 8.5 Å are found for full agonists of low potency relative to 5-methylfurmethide. Indeed, the less potent agonists (*S*)-QNAH, MSDQ, and (*R*)-QNAMe have P to terminal methyl distances of 7.5, 7.8, and 8.1 Å, respectively. In the case of (*R*)-QNAMe the lower potency may well arise from a combination of $|PQ|$ and $|PC_t|$ both smaller than the optimal pharmacophore values.

The Active Conformation of Acetylcholine. For ACh and β-MeACh, the muscarinic pharmacophore can be attained for any τ_1, τ_2 pair on the line segment labeled 115° in Figure 4 and lying between its two points of intersection with the 3 kcal/mol contours. One of these end points, referred to as the *A* conformer, with $\tau_1 = 189°$ and $\tau_2 = 132°$, has $|PC_t| = 8.5$ Å. The second conformer, *B*, has $\tau_1 = 260°$, $\tau_2 = 78°$, and $|PC_t| = 6.5$ Å. Both conformers are shown in Table II for ACh and β-MeACh. From the previous discussion of 5-methylfurmethide and the other semirigid agonists, it seems clear that *the conformation of ACh and β-MeACh employed in the muscarinic pharmacophore is the A conformation* ($\tau_1 = 189°$, $\tau_2 = 132°$).

Related to ACh is propionylcholine, a full agonist less potent than ACh. Use of the *A* conformation for this agonist with one additional methylene group is not possible, since each methylene lengthens the $|PC_t|$ distance by 1.2 Å (for an elongated alkyl chain), and $|PC_t|$ would then be 9.9 Å. However, by using a conformation on the 115° line of Figure 4 between *A* and *B*, a $|PC_t|$ value of 8.5 Å can be obtained. By moving further toward *B*, it may be possible to accommodate the two additional methylene groups of butyrylcholine, which is a partial agonist on the rat jejunum. On the other hand, the three additional methylenes of valerylcholine probably lengthen the chain too much to enable it to fit into the receptor cavity. In other words, for the choline esters, and presumably the ethers as well, there is not a single conformation used to attain the pharmacophore geometry, but rather a range from which τ_1 and τ_2 values are selected according to the size of the acyl group. The existence of a range of accessible pharmacophore conformations also explains the occurence of muscarinic agonists having head groups of varying size—either larger alkyl groups on the nitrogen or replacement of the nitrogen by phosphorus or arsenic.

The reason for the decreased potency of propionyl- and butyrylcholine relative to ACh is not fully clear. It may be due to the tighter fit to the binding site and the smaller $|PQ|$ value associated with the *B* conformation, 6.5 Å, Table II. It also may be related to the fact that the carbonyl oxygen in the *B* conformation is in a quite different position from that in the *A* conformation, and the latter more closely resembles the location of the hydroxy oxygen of muscarine and the π-electron system of 5-methylfurmethide.

Comparison of the Active and X-ray Conformations of Agonists. The particular dihedral angles employed by each agonist define its pharmacophore geometry. Since there is a large literature concerned with these angles, we give in Table III X-ray and pharmacophore dihedral angles for the agonists considered in the present work. For ACh there are two sets of X-ray angles corresponding to the crystalline bromide and chloride, i.e., gauche–gauche and trans–gauche geometries. The column labeled "active conformer" contains the τ values corresponding to the conformers *A* and *B*. It is interesting to note that neither of these corresponds to the X-ray value, nor do they correspond to the trans–trans geometry, another often-considered local minimum on the ACh surface.

Table III. Comparison of X-ray τ_1 and τ_2 Values for Muscarinic Drugs with Those in the Active Conformation

agonist	X-ray		active conformer	
	τ_1	τ_2	τ_1	τ_2
ACh	79 (193)[a]	77 (85)	189 (260)[b]	132 (78)
β-MeACh	217	87	189 (260)[b]	132 (78)
muscarine	144	73	159	143
F2268	103	94	158	140
5-methylfurmethide	174	83	175	125
ACTM	147	137	150	146
(*R*)-QNAMe	76	106	187	122

[a] Values for the bromide and chloride (in parentheses). [b] The two sets of τ values correspond to the extreme conformations consistent with the pharmacophore.

Similarly, there is little coincidence between the X-ray and active conformer dihedral angles for the semirigid agonists. Indeed, the discrepancy is very large for (*S*)-QNAH [X-ray values inferred from those of (*R*)-QNAMe]. The case where there is most agreement between crystal and pharmacophore geometry is ACTM, with less than 10° difference between the two sets of dihedral angles. Since this crystal geometry closely resembles that of the pharmacophore, it was used to construct the conformation depicted in Figure 2. The contents of Table III demonstrate that a search for a consistent pattern of crystal torsional angles cannot reveal the nature of the muscarinic pharmacophore.

Discussion

Through consideration of both drug and receptor sites and extensive use of quantum mechanical calculations for accurate conformational energies, the model has identified the muscarinic pharmacophore, interpreted aspects of agonist stereoselectivity in the QNA series, and quantified the relationship between chain length and partial agonism and antagonism.

It might be noted that little mention has been made of the possible role played by the carbonyl oxygen, except in discussing the B conformer and ACh. It has not been excluded from participation, because the muscarinic pharmacophore involves active conformations that place the negative potentials associated with the carbonyl group of the esters, the second ether oxygen of F2268, the hydroxy of muscarine, and the π electrons of 5-methylfurmethide in similar spatial positions. Thus, this position is already determined by the two parameters of the model and is redundant in identifying the pharmacophore. This fourth site is quite probably necessary for receptor recognition and activation. As mentioned previously, the lower potency of propionyl- and butyrylcholine, which probably use the B conformation, may well be ascribed to the placing of the carbonyl oxygen in a different position.

The present work has interpreted the lower potency in the QNA series to a lesser ability of the agonist to adopt the muscarinic pharmacophore geometry, e.g., the correct $|PQ|$ or $|PC_t|$ value. Within the context of Stephenson's extension of the occupancy theory,[21] a better approximation to the ideal pharmacophore would result in a higher efficacy. Still, the mechanism by which the agonist activates the muscarinic receptor is unclear, and the detailed roles of the head group, the terminal alkyl group, and the potential minimum at *Q*, if other than binding, remain

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unknown. The transition from full to partial agonism or antagonism has been related to increased size of the terminal group as monitored by the $|PC_t|$ value. To speculate, this could arise from the ability of a partial agonist to bind both to a site furnishing the activity and to a null or antagonist site. The extent of the partitioning between the two sites would depend on the relative binding constants, a function of chain length, and other parameters, such as stereochemistry, hydrophobicity, etc., of the terminal group. Antagonists then, are drugs that are too large to fit into the active site and that bind entirely, and indeed strongly, to the null site.

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Registry No. a, 51-84-3; b, 55-92-5; c, 21408-92-4; (*S*)-e, 84863-70-7; g, 300-54-9; h, 58316-49-7; i, 14172-53-3; (*R*)-QNAMe, 82264-25-3; MSDQ, 84863-71-8.

α-Adrenoreceptor Reagents. 1. Synthesis of Some 1,4-Benzodioxans as Selective Presynaptic α_2-Adrenoreceptor Antagonists and Potential Antidepressants

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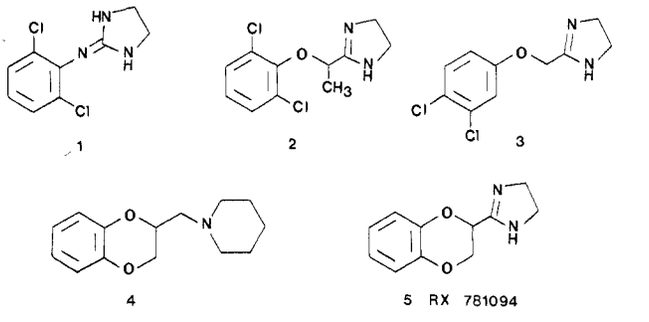
The rational design of RX 781094, 2-(1,4-benzodioxan-2-yl)-2-imidazoline hydrochloride (**5**), a new potent and selective antagonist of α_2-adrenoreceptors, is discussed. A compound that acts as an antagonist at presynaptic α_2-adrenoreceptors could be an effective and novel treatment of depression because of its ability to increase the concentration of norepinephrine at central receptor sites. The effects of substituents in the aromatic and imidazoline rings have been examined, as well as the replacement of the imidazoline ring by an amidine function or by other heterocyclic ring systems. None of these derivatives are as potent or selective as **5**, although some do display a degree of selectivity as antagonists. Some derivatives were found to possess agonist properties that, with the exception of **23**, favored the postsynaptic site. Compounds **9**, **12**, **16**, **21**, **30**, and **51** possessing presynaptic α_2-adrenoreceptor antagonist and postsynaptic α_1-adrenoreceptor partial agonist properties were also obtained, and these derivatives could be considered as potential antimigraine agents.

According to the catecholamine hypothesis of affective disorders, a relative deficiency of the transmitter norepinephrine at receptor sites within the central nervous system is responsible for the symptoms of the disease. Support for this hypothesis is provided by drugs that are clinically effective antidepressants and that increase the concentration of norepinephrine at central receptor sites.

In the early 1970's the accepted concepts of noradrenergic transmission were challenged by a hypothesis that proposed that the release of norepinephrine was regulated by presynaptic α-adrenoreceptors (negative-feedback mechanism[1]). It was proposed that these presynaptic α-adrenoreceptors should be designated the prefix α_2 in order to differentiate them from postsynaptic α-adrenoreceptors, which were themselves designated α_1. However, recent evidence suggests that α_1- and α_2-adrenoreceptors are general subtypes, and the term α_2 should not be limited to presynaptic receptor sites.[2]

Recently, it has been proposed that a common underlying mechanism of action of antidepressants may be an ability to alter α_2-adrenoreceptor sensitivity.[3] Thus, in depression these receptors (α_2) are considered to be supersensitive, hence, turning off release of norepinephrine. It has been suggested[3] that a feature of antidepressant therapy is a gradual reduction in the sensitivity of central α_2-adrenoreceptors: such a subsensitivity would gradually increase norepinephrine levels. Therefore, it can be proposed that if central α_2-adrenoreceptor subsensitivity is a prerequisite for onset of antidepressant effect, then a compound that acts as an antagonist at these receptors could be an effective and novel treatment of depression. At present, yohimbine is used pharmacologically as a selective α_2-adrenoreceptor antagonist, but its lack of specificity limits clinical application.

Clonidine (**1**) is an α_2-adrenoreceptor agonist used clinically as an antihypertensive. Clonidine and a series of aminoimidazolines, differing only in the choice of aromatic substituents, were previously profiled in vitro for α_2/α_1 selectivity and agonist and antagonist character.[4] It



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