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Review Article

Structural requirements of muscarine, 1,3-dioxolane and their analogues for agonist activity at cholinergic receptors

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Introduction

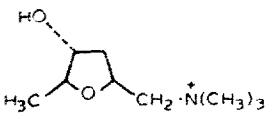
Structure–activity studies have been used extensively in investigations to define the 'active' conformation of cholinergic molecules in the search for information about the molecular nature of cholinergic receptors and the mechanism of interaction of agonists, and antagonists with the receptors. Since the early 1960s an enormous amount of work has been devoted to the synthesis and pharmacological examination of a large number of analogues and congeners of acetylcholine, muscarine and 1,3-dioxolane. Gualtieri et al. (1979) pointed out the drawbacks: (a) in using structurally induced variations in the receptor effectors; (b) the determination of solid state conformations and preferred conformations in water of active ligands; and (c) the use of conformationally-restricted analogues of acetylcholine, in the efforts to obtain information on the molecular environment of the muscarinic and nicotinic sites. They concluded that the use of molecular structures which are sufficiently rigid to allow for only one probable conformation, even after major structural modifications, and which also display significant activity, would eliminate these drawbacks. This review is confined to the investigations which have been conducted using analogues of muscarine and 1,3-dioxolane for determination of the structural requirements for agonist activity at cholinergic receptors.

Muscarine and its analogues

Structural requirements for agonist activity

There are 4 pairs of enantiomers of muscarine, of which the most active is the naturally occurring compound, L-(+)-muscarine (2S, 3R, 5S) (1).

The stereoselectivity exhibited by the L-(+)-isomer is shown in the comparative data presented in Table 1. While L-(+)-muscarine is not the most active muscarinic



(1)

agent known, it is probably one of the most specific, being devoid of nicotinic activity. It is also evident from Table 1 that the 2-methyl group of muscarine plays a significant role in the control of muscarinic activity since its removal, as in 2-desmethylmuscarine, leads to a very significant decrease in activity; the addition of a second 2-methyl group gives an even greater reduction in activity.

In contrast to the high degree of stereospecificity shown by the cholinergic receptor towards the muscarine isomers, the interaction between the receptor and muscarone and related compounds is relatively non-stereoselective. The more potent isomer of muscarone has the D-configuration, which is an inversion of the stereospecificity of action found in the muscarines. The muscarones also exhibit significant nicotinic activity (Gyermek and Unna, 1958; 1960; Waser, 1961).

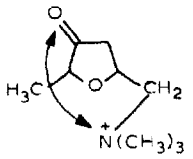
Proposed models for binding to cholinergic receptors

Several workers who conducted comprehensive stereochemical and pharmacological studies of the muscarine and muscarone series in the early 1960s proposed essential structural features for binding to receptor sites. Beckett et al. (1961) proposed that the muscarinic receptor has two main centres for drug–receptor association, an anionic cavity to accommodate the quaternary nitrogen group and a positively-charged site to interact with the ether oxygen of muscarine. The –OH group at position 3 is considered to act as a secondary site of association. Waser (1961) postulated ionic binding of the quaternary nitrogen, and hydrogen binding to the ether oxygen atom at a distance of 3.8 Å, slight binding of the –OH group in the muscarines, and strong binding of the C_2-methyl group by van der Waal's forces. He

TABLE 1
CHOLINOMIMETIC ACTIVITIES OF MUSCARINE AND RELATED COMPOUNDS (GYERMEK AND UNNA, 1958; WASER, 1961)

Compound	Cat b.p.	Equiactive molar ratio (ACh = 1)		
		Frog heart	Rabbit ileum	Frog rectus
DL-muscarine	0.75	10	1.0	> 50
L-(+)-muscarine	0.32	5	0.33	> 50
D-(−)-muscarine	350.0	–	130.0	> 50
DL-epi-muscarine	230.0	> 1000	230.0	> 200
DL-allo-muscarine	130.0	> 1000	150.0	> 200
DL-epi-allo-muscarine	75.0	> 1000	220.0	> 100
DL-2-desmethylmuscarine	80.0	> 1000	–	–
DL-2-desmethyl-epi-muscarine	550.0	> 1000	–	–
DL-2-methylmuscarine	650.0	> 1000	–	–

explained the anomalous high potency of D-(−)-muscarone by proposing that the pharmacologically active conformation of the D-(−)-isomer would be as shown in (2), in which the carbonyl oxygen is considered equivalent in the D-isomer to the ether oxygen of the L-(+)-isomer. He also suggested that the carbonyl oxygen would

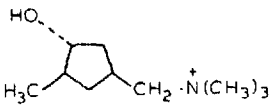


(2)

now be responsible for hydrogen bonding with the receptor to produce muscarinic activity. Belleau and Puranen (1963) found serious difficulties with this interpretation as the proposed active conformation (2) would be of very high energy because of the many unfavourable non-bonded interactions, and also in that it is a complete departure from the maximum coplanarity which is almost uniformly necessary for effective binding of the atoms contributing to binding. They suggested that conformation (2) for D-muscarone is unlikely and believed that a more likely possibility is that the carbonyl group in muscarone interacts with an accessory nucleophilic site on the receptor, thus introducing an additional factor contributing to binding in a unique manner. On the basis of several independent lines of evidence, Moran and Triggle (1971) proposed the concept of multiple ligand binding modes centred around a common functional or catalytic centre, and separate characters of the agonist and antagonist binding sites at muscarinic receptors. Subsequently, Triggle and Triggle (1976) suggested that there may be at least two binding subsites for agonists at these receptors, a polar area of high stereoselectivity, and a non-polar site with low steric demand.

Cyclopentane analogues of muscarine and muscarone

Replacement of the ether oxygen by an isosteric unit, a methylene group, whose electronic properties differ radically from those of the ether oxygen was carried out by Sundelin et al. (1973), who synthesized (3) and (4), which are cyclopentane analogues of muscarine and deoxamuscarine, respectively.



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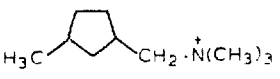
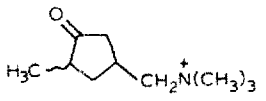


(4)

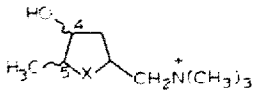
The racemic cyclopentane muscarine analogue (3) is 5–10 times more active than acetylcholine and muscarine in guinea pig ileum. However, the deoxy cyclopentane derivative (4) has only about one-hundredth the activity of acetylcholine. The unusually high activity reported for (3) suggested that the role of the ether oxygen as an electron-rich centre is not as critically important as a primary site of interaction as had earlier been assumed. Gualtieri et al. (1974) synthesized the racemic form of

the cyclopentane analogue of muscarone, 4-methyl-3-oxo-1-dimethylaminomethyl-cyclopentane (5), and found that it had about the same activity as acetylcholine, when assayed on 4 in vitro muscarinic preparations, guinea pig terminal ileum, rat jejunum, guinea pig ductus deferens and guinea pig auricles. They were unable to isolate the *cis*- and *trans*-isomers because of the rapid isomerization of the racemic compound. Simultaneously, Givens and Rademacher (1974) reported on the synthesis of (±)-(5), and compared its muscarinic and nicotinic properties with those of



(5)

(3). The muscarinic activity of the ketone was found to be significantly higher than that of the alcohol in the desether series. Nicotinic activity of (±)-(5) using the chick biventer cervices preparation was significantly greater than that of the alcohol, indicating a pronounced change in nicotinic activity with oxidation to the ketone. These results suggested that the role of the ether oxygen is not one of primary binding, although it may be exerting a secondary influence, the nature of which is uncertain. A suggested possible role could involve increased hydrophilic bonding at the membrane surface permitting enantiomer recognition at the receptor site. The cyclopentane analogs of muscarine and its enantiomers (deoxamuscarines) (6–9) were synthesized and tested on a number of cholinergic preparations in order to obtain further clarification of structure–activity relationships of cholinergic compounds (Gualtieri et al., 1975; Melchiorre et al., 1975a and b; Gualtieri et al., 1976; Melchiorre et al., 1977).



(6) X = CH_2 ; 4t,5c
(7) X = CH_2 ; 4c,5c
(8) X = CH_2 ; 4c,5t
(9) X = CH_2 ; 4t,5t

The potencies of the deoxamuscarines, expressed as equipotent molar ratios with reference to acetylcholine, were compared with those for the corresponding isomers of muscarine and its enantiomers. The authors reached the following conclusions on the comparative results.

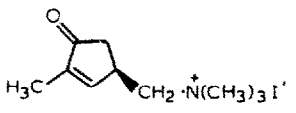
(1) The pattern of the activities of the muscarine and the deoxamuscarine series is quite similar, suggesting that steric relationships play a fundamental and similar role in both series.

(2) The muscarinic activity of the deoxamuscarines is generally less potent than that of the corresponding muscarines. However, the difference in potency between deoxamuscarine and the less potent isomers is smaller, suggesting that the ether oxygen, although not crucial for activity, is a factor contributing to specificity.

(3) The deoxamuscarines showed different potencies on the different tissues used.

More recently, Angeli et al. (1981) have provided further evidence in support of the hypothesis of a duality of binding sites for ligands at cholinergic receptors. They synthesized the methiodides of 2,3-dehydrodeoxamuscarone (10), *cis*-2,3-dehydrodeoxamuscarine (11) and *trans*-2,3-dehydrodeoxamuscarine (12), and compared their

muscarinic and nicotinic activities with those of 3-methyl-1-(N,N-dimethyl-aminomethyl)cyclopent-2-ene methiodide (13). The latter is one of the most active cyclopentane derivatives of muscarine which lack an oxygen function (Giannella et al., 1980).



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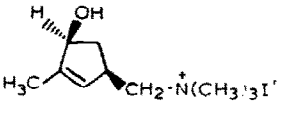

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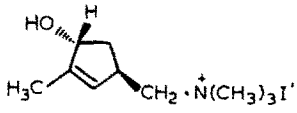

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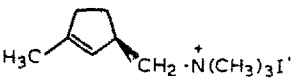
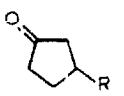


(13)

The muscarone derivative (10) had improved muscarinic and nicotinic activities compared with (13). Specificity as far as muscarinic activity is concerned is low; activity was 10 times lower than that of the corresponding muscarones, but nearly equivalent to that of deoxamuscarone (Melchiorre et al., 1975a). In the case of *cis*-2,3-dehydrodeoxamuscarine (11), there was a drop of two orders of magnitude in both muscarinic and nicotinic activities. A small decrease in muscarinic activity was observed with the *trans*-isomer (12), but the specificity was greatly increased since nicotinic activity was practically absent. This isomer had a pattern of activity similar to that of muscarine. Since the *trans*-isomer was found to be less active than deoxamuscarine as a muscarinic agent, the planar configuration of the methyl group at position 2 imposed by the double bond, appears to be of some relevance in this case, unlike the corresponding carbonyl compound (10). The authors concluded that the dramatic change in the biological profile of the molecule, following introduction of the hydrophilic hydroxyl group, lends support to the hypothesis of Triggle and Triggle (1976).

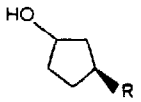
Further evidence supporting the "5-atom" rule

Early studies of the structural requirements for muscarinic activity led to the proposal of the "5-atom" rule, whereby maximum agonist activity is associated with an effective 5-atom chain length attached to the quaternary nitrogen function. This rule is applicable to cyclic and open-chain compounds which are active at muscarinic receptors. The 5-atom chain always terminates in a methyl group, and it is believed by some workers that the latter is necessary for correct binding at muscarinic receptors. It has been mentioned earlier in this review that removal of the terminal methyl group of muscarine, to give 2-desmethylmuscarine, leads to a very significant decrease in activity. Similar results have been found with the structurally related 1,3-dioxolanes, which will be discussed later. Melchiorre et al. (1975c) synthesized the cyclopentane analogues of desmethylmuscarone (14), epi-desmethylmuscarine (15a) and desmethylmuscarine (15b), and compared their activities at muscarinic and nicotinic receptors with those of deoxamuscarone and the corresponding de-



(14)

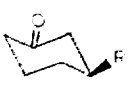
$R = CH_2 \cdot \overset{+}{N}(CH_3)_3 I^-$



(15a) 3-OH cis
(15b) 3-OH trans

oxamuscarines. The muscarinic activities of (14) were from 100 to 1000 times lower, and the nicotinic activity 13 times less than those for deoxamuscarone. The authors also reported a sharp drop in activity at both muscarinic and nicotinic receptors for (15b) as compared with deoxamuscarine (the comparison using guinea pig tracheal muscle was an exception). The nicotinic activities of (15a) were weaker than those of allo- and epi-deoxamuscarines, and the desmethyl derivative was also less active at 4 of the 6 muscarinic receptors used. These results provide additional evidence in support of the 5-atom rule.

Angeli et al. (1977) synthesized the cyclohexane analogues of deoxamuscarine (16–18).



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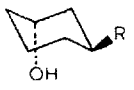

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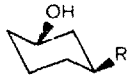


(18)

$R = CH_2 \cdot \overset{+}{N}(CH_3)_3 I^-$

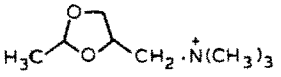
These analogues represent structural alterations of deoxamuscarine and deoxamuscarone in that the methyl substituent of the cyclopentyl compounds forms part of the enlarged ring, and the '5-atom chain' is retained.

Muscarinic activity, measured on guinea pig terminal ileum, showed a large decrease when compared with the cyclopentyl analogues. The authors established the configuration of the cyclohexane analogues, and found that the difference in the relative positions of the oxygenated functions and the quaternary group of the two cyclic systems is rather small, so that the major difference is in the relative arrangements about carbon 7 (4-methyl in the cyclopentyl, and methylene (position 4) in the cyclohexyl series). This observation provides further confirmation of the importance of the 4-methyl group in the deoxamuscarines and deoxamuscarone for muscarinic activity.

1,3-Dioxolane and its analogues

The 1,3-dioxolane analogues also illustrate the stereochemical requirements for activity at muscarinic receptors. 2-Methyl-4-dimethylaminomethyl-1,3-dioxolane (19) has been shown by Triggle and Belleau (1962) to be an active stereoselective agonist at muscarinic receptors. The *cis*-isomer is about 5–10 times more potent than the

trans-isomer (loc. cit.) and 2S, 4R (L-*cis*)- is approximately 100 times more active than the 2S, 4S (D-*cis*)-isomer (Belleau and Puranen, 1963).



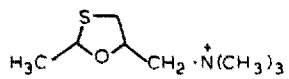
(19)

Contribution of the 2-methyl group to agonist activity

The contribution of the 2-methyl group to the high agonist activity of the (L-*cis*)-isomer is much greater than that predicted by the π-value, and may indicate a highly productive interaction of this ligand with the receptor relative to other members of this series (Chang et al., 1972). Absence of the 2-methyl group gives rise to an approximately 1000-fold loss in activity, and the presence of a second methyl group at position 2 also reduces activity. Substitution of the 2-methyl group in (19) by phenyl gave a compound with antagonist activity at muscarinic receptors; the *cis*- and *trans*-isomers were not, however, significantly different in activity (May et al., 1969). Subsequently, Brimblecombe and Inch (1970) found that derivatives of 4-dimethylaminomethyl-1,3-dioxolane with two bulky substituents at C-2, e.g. benzyl and cyclohexyl, had anticholinergic properties similar to those of atropine in the peripheral nervous system. Studies by Chang et al. (1972) have shown that, in contrast to the highly stereoselective and restrictive binding of potent agonists, such as (19), at the neurotransmitter recognition site, interaction may become increasingly more difficult and less effective at this site, with progressive incorporation of non-polar groups at C_2. Incorporation of the rigid 2-aryl or 2-cyclohexyl substituents precludes interaction at this site, and re-orients ligand binding to a predominantly non-polar subsite. The effects of the C_2-methyl group on muscarinic activity appear to be similar in the 1,3-dioxolanes and muscarines. The relative lack of configurational activity in the *cis*- and *trans*-isomers of 2-methyl-4-dimethylaminomethyl-1,3-dioxolanes is more similar to the situation with the muscarones than the muscarines, but the chirality of the interaction of the dioxolanes with muscarinic receptors is the same as that of the muscarines.

Cholinergic activities of related oxathiolanes and dioxolane analogues

In order to obtain information about the contribution of the oxygen at position 1 of *cis*-1,3-dioxolane (19) to the cholinergic activity, Elferink and Salemink (1975) synthesized the *cis*-isomer of oxathiolane (20), and compared the muscarinic activity, using the rat jejunum, with that of (19) and of dithiolane (21). The results are shown in Table 2



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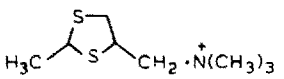
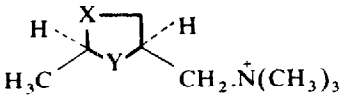


(21)

The very high muscarinic activity of 20 was unexpected, as it had been considered by earlier workers (Beckett et al., 1961; Waser, 1961) that interaction of the keto

TABLE 2
MUSCARINIC ACTIVITY OF COMPOUNDS OF THE FORMULA

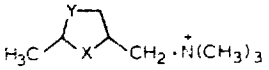


	X	Y	Muscarinic activity		
			pD_2	Equimolar dose	α
(19)	O	O	6.9	0.7	1
(20)	S	O	7.0	0.5	1.2
(21)	S	S	4.7	120	1

Acetylcholine was used as a standard with $pD_2 = 6.7$ and intrinsic activity (α) = 1. The authors found that the dose–response curves of the oxathiolane and the dithiolane were not parallel with those of acetylcholine, but were steeper.

group in acetylcholine and muscarone, the hydroxyl group in muscarine and the oxygen at position 1 in dioxolane with the receptor, was by hydrogen bond formation. However, this type of bond with the receptor is less likely in the case of the oxathiolane (20), because of the weaker ability of sulphur to form a hydrogen bond.

More recently, Gualtieri et al. (1979) investigated the activities of dioxolane analogues by isosteric substitution of the oxygen at 1 and 3, respectively, by a methylene group into *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (19) to give compounds (22) and (23).



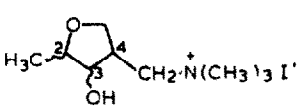
(22) Y = O , X = CH_2
(23) Y = CH_2 , X = O

Comparisons of activities at muscarinic receptors, showed that, except for the effect on guinea pig ileum, compounds (22) and (23) are only slightly less active than (19). Apparently, therefore, substitution of oxygen 1 or 3 in (19) by a methylene group does not substantially alter the muscarinic activity of the molecule in some preparations. The respective roles of the two oxygens are apparently quite different, as far as nicotinic activity is concerned. Substitution of oxygen at position 1 by methylene (22) reduces activity, whereas a similar substitution at position 3 (23), causes a slight increase. The authors showed that isosteric substitution of the carbonyl group of acetylcholine by methylene does not greatly diminish muscarinic activity, whereas substitution of the ether oxygen by methylene causes a substantial loss of activity at muscarinic receptors.

Isomuscarines

In an effort to differentiate between the roles of the two oxygenated functions,

Gualtieri et al. (1979) synthesized and tested compounds in which the oxygen in position 3 of dioxolane was substituted by a CHOH group. The resulting compounds (24–27) have the oxygenated functions inverted with respect to muscarine (isomuscarines).



(24): 4r, 2c, 3t
(25): 4r, 2c, 3c
(26): 4r, 2t, 3c
(27): 4r, 2t, 3t

None of these compounds had muscarinic activity (guinea pig ileum) or nicotinic activity (frog rectus abdominis muscle). The authors explained this result by assuming a dipole—dipole interaction between the receptor and the oxygen in position 3 of *cis*-dioxolane (19), the orientation of the receptor dipole being strictly limited and parallel to the plane of the ring. When a CHOH group is substituted for the ether oxygen at this position, the dipole direction changes according to the orientation of the OH group, and is no longer in the plane of the ring, thus preventing proper drug–receptor interaction.

Dipole–dipole mechanism for interaction at muscarinic receptors

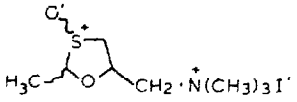
Comparison of the potencies of a number of compounds obtained by modifying the dioxolane molecule in positions 1, 3 or both, led Gualtieri et al. (1979) to propose a dipole–dipole interaction at the muscarinic receptor sites binding the oxygens at positions 1 and 3. While the site corresponding to oxygen 1 can accommodate groups of different size and polarity, e.g. OH and S, without a significant change in potency, the site corresponding to oxygen 3 cannot accept groups which are larger or with a dipole having a different direction than the ether oxygen, e.g. CH_3, OH or S. These authors suggested that the dipole–dipole interaction provides a better explanation for the high potency of the oxathiolane (20) than hydrogen bonding. A schematic representation of the active site of the muscarinic receptor was proposed by Gualtieri et al. (loc. cit.) based on the inferences drawn from the results of their investigations. This model differs from that proposed by Beckett (1967) in two principal features: (i) the driving forces for interaction are those resulting from the interaction of the onium and methyl groups, whereas Beckett proposed those deriving from the onium and the oxygenated functions; and (ii) a dipole–dipole interaction binding the oxygens at the 1 and 3 positions of dioxolane, whereas Beckett suggested hydrogen bonding at both these positions.

Oxathiolane sulphoxides and sulphones

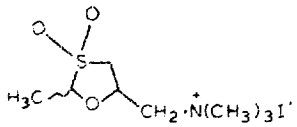
The hypothesis of a dipole–dipole interaction was further investigated by Pigini et al. (1981). They synthesized and tested 2L-methyl-5r-dimethylaminomethyl-1,3t-oxathiolane sulphoxide methiodide (28a) and its 3 isomers (28b–d) in which a strong, oriented dipole was present. The sulphone analogues of (19) (29a and b) were also synthesized and tested.

The *trans*-form of 2-methyl-5-dimethylaminomethyl-1,3-oxathiolane methiodide

was also synthesized. The *cis*-isomer (20) was described earlier (Elferink and Salemink, 1975)



(28a): 2c,3t,5r
(28b): 2t,3c,5r
(28c): 2t,3t,5r
(28d): 2c,3c,5r



(29a): cis
(29b): trans

The authors (loc. cit.) found that the pattern of muscarinic activity of compounds (28a–d) on guinea pig ileum is generally similar to that of muscarines and deoxamuscarines. Since the pharmacophoric groups (2-methyl, 5-onium and nuclear oxygen) are identical in (28a) and in muscarine, and since their activities are almost identical, it was concluded that the fourth group (S^+-O' and CHOH, respectively) binds to the active site of the receptor in the same way. The corresponding sulphone (29a), which differs only in the size of the group and in the direction of the dipole, was found to be 150 times less potent than (28a). This evidence provides confirmation of the importance of the size of the group and the strength and direction of the dipole at position 3 for binding to the receptor. The S^+-O' group is, however, capable of binding as a hydrogen bond "acceptor". The sulphoxide (28a) was found to be nearly 20 times less potent than the *cis*-isomer of (20) and slightly less specific. These results conflict with both the hypothesis of hydrogen bonding (Beckett, 1967) and that of dipole–dipole interaction at the muscarinic site of the receptor, since in either case the fit of (28a) with the receptor should be better than that of (20). Pigini et al. (1981) have pointed out that the activity of *cis*-oxathiolane (20) is exceptional in this class of compounds, being about twice as potent as (±-muscarone) and approximately 20 times more potent than acetylcholine. The *cis*-oxathiolane is about 100 times more potent than its *trans*-isomer, one order of magnitude more than in the corresponding dioxolane series. The authors contrasted the conflicting evidence obtained on comparing the muscarinic activities of (28a) and (20) with that observed by Angeli et al. (1981) for cyclopentane analogues of deoxamuscarines and deoxamuscarones and suggested that (28a) and (20) might interact with different subsites of the muscarinic receptor. Equipotent nicotinic activities were observed for (28a) and (28d), and the sulphone (29a) was found to be more active as a nicotinic agent than the sulphoxides. The *cis*-oxathiolane (20) also exhibited good nicotinic activity.

Further studies are necessary to establish the precise steric and polar requirements for agonist activity at muscarinic and nicotinic receptors, and thus provide information on the active sites of the receptors. The results of the recent studies using analogues of the muscarinic effectors muscarine and 1,3-dioxolane have contributed substantially to the knowledge of structural requirements for agonist activity at cholinergic receptors. These results complement current studies on the

nature and structure of these receptors, and the collective information should advance the knowledge of the chemical nature of the receptors, and of the mechanisms of their interaction with ligands.

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