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RIGID ANALOGS OF ACETYLCHOLINE CAN BE M1-SELECTIVE AGONISTS: IMPLICATIONS FOR A RATIONAL TREATMENT STRATEGY IN ALZHEIMER'S DISEASE

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Abstract

Rigid analogs of acetylcholine offer an opportunity for selective actions at muscarinic receptor subtypes, since restricted conformational mobility alters the capacity of ligands to adapt to subtle differences in receptor structure. **AF102B**, a highly rigid analog of acetylcholine, is a centrally active M1 agonist and is evaluated in light of some currently available therapeutic strategies in Alzheimer's disease.

Introduction

Molecular biology has revealed the existence of five distinct human muscarinic receptor (mAChR) subtypes (m1-m5)[1]. It is likely that the m1, m2, m3, m4 AChRs fit the pharmacological definition of the M1, M2, M3 and M4 AChRs, respectively[1]. According to the cholinergic hypothesis in Alzheimer's disease (AD)[2], the demented state might be alleviated by treatment with M1 or m1 agonists[3], M2 or m2 antagonists[3a,b], m3 agonists[3b], or m3 antagonists[3c,**].

An implicit assumption, in the search for M1 or m1 specific muscarinic agonists, has been that there are differences in the binding sites for the mAChR subtypes[1,3]. In this regard, it has been suggested that for rigid analogs of acetylcholine (ACh), the conformational and stereochemical restraints of a particular mAChR subtype can uniquely be satisfied while others are partially excluded for the other receptor subtypes[4]. In addition, rigid ligands can display different receptor association and dissociation characteristics, when compared with analogous flexible structures[5]. This may result in selectivity which has nothing to do with the specific pharmacophoric pattern of the rigid ligand. Moreover, selectivity may not result from the agonist/receptor interaction alone, but also from the properties of the agonist/receptor/G-protein(s) complexes. Those questions will be addressed through examination of the structural and pharmacological profile of (±)*Cis*-2-methyl-spiro(1,3-oxathiolane-5,3')quinuclidine, **AF102B**, which is a selective M1 agonist[3a,4a-c,6-9]. In this paper some current features of **AF102B**, which may be relevant to a rational treatment strategy in AD are presented. Comparison is made, whenever possible, with some new and old muscarinic agonists (Fig 1).

Materials and Methods

Chinese hamster ovary (CHO) cells stably transfected with rat m1 AChR or m3 AChR and human m1-m5 AChRs were used in this study. In these cell cultures tested agonists were assayed on the following parameters and according to the methods in cited refs: ***phosphoinositide (PI) hydrolysis*** [1b]; ***cyclic AMP accumulation*** [1b]; ***arachidonic acid mobilization*** [1c]; ***intracellular Ca^{2+} accumulation*** [1d].

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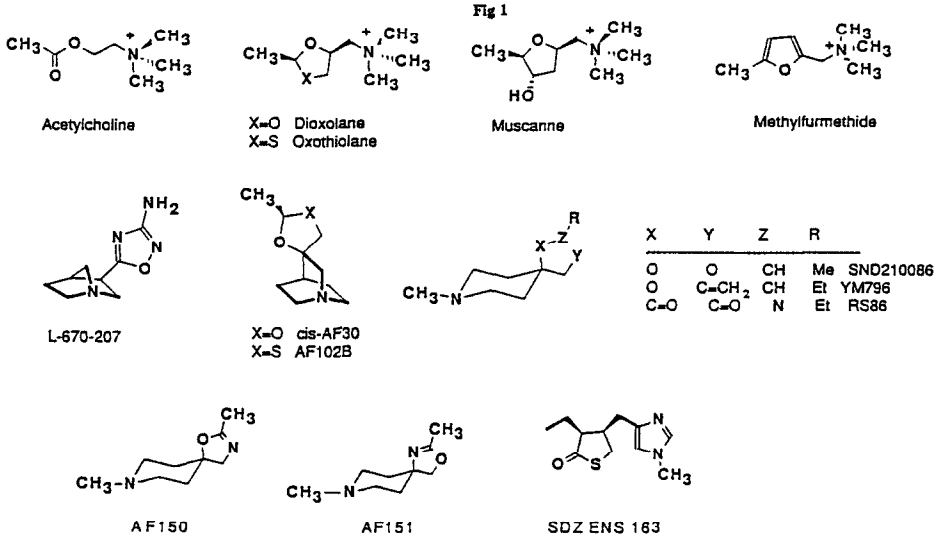
* To whom correspondence should be addressed. ** The terms m1, m2, m3, m4 or m5 and M1, M2 or M3 ligands (agonists or antagonists) are used for ligands defined using the cloned m1-m5 and M1, M2 or M3 pharmacologically characterized mAChRs, respectively.

Fig 1



Results and Discussion

The findings obtained to date indicate that **AF102B** is a rather selective M1 agonist, exhibiting unique properties in restoring memory impairments in a variety of animal models for AD[3a,4,6-9]. Notably, **AF102B** can act as a full agonist, a partial agonist, or an antagonist depending on the tissue, on the mAChR subtype and functional assay studied. Examination of Table 1 and the cited references[3a,4,6-9] indicates that **AF102B** can best be considered as a selective M1 or m1 agonist when defined through binding[3a] and functional[3a,4,6,8,9] assays. Yet, it is possible that due to different receptor reserves, **AF102B** might show full agonistic activity on certain M1 AChRs (e.g. those modulating memory processes) and partial agonistic or even antagonistic activity on other type of functions mediated by peripheral or central (M1, M3?) AChRs[3a,4a-c,6-9]. Albeit plausible, this explanation cannot clarify completely why **AF102B** does not induce mainly central effects such as tremors, hypothermia and purposeless chewing (M2 or M3 agonistic effects)[4a,7]. In fact, the M2 are more efficiently coupled to effector systems than the M1 AChRs[4d]. Thus, a more reasonable explanation would be that **AF102B** is a unique functionally selective M1 and m1 agonist. In this context, in CHO cells transfected with m1 AChR, **AF102B** increased intracellular Ca^{2+} almost to the same extent as carbachol, while activating only partially PI hydrolysis and arachidonic acid release, and completely failing to increase cAMP accumulation (Table 1). By comparison, **AF102B**, showed only marginal agonistic effects on PI hydrolysis and arachidonic acid release and no effect on cAMP accumulation in CHO cells transfected with the m3 AChR subtype. Results obtained with the m2, m4 and m5 AChRs also emphasize the functional selectivity of **AF102B** towards m1 AChRs (Table 1).

Table 1. The effects of McN-A-343*, AF102B, AF150 and AF151 on PI hydrolysis, arachidonic acid release, cAMP accumulation and increase in intracellular Ca^{2+} levels in CHO cells stably transfected with human m1-m5 AChRs. Results in % are expressed relative to carbachol-induced effects.

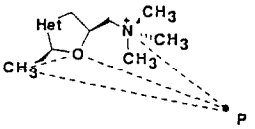
human mAChR in CHO cells	Assay	McN-A-343	AF102B	AF150	AF151
m1	*PI hydrolysis*	89±3	43±5	85	102
	AA release	90±9 (6)	68±13 (8)	90±5 (3)[a]	61[a]
	cAMP accumulation	7, 5 (2)	1±0.4 (4)	6	3
	$[Ca^{2+}]i$ increase	*nd*	79	60	85
m2	*cAMP decrease*	9.5±5 (3)	60±6 (8)	74	70
	$[Ca^{2+}]i$ increase	*nd*	17	12	9
m3	*PI hydrolysis*	7±1 (3)	10±2 (5)	76	84
	AA release	13±2 (6)	11±3 (8)	27±4 (3)[a]	36[a]
	cAMP accumulation	7, 0 (2)	0.8±0.4 (4)	10	4
	$[Ca^{2+}]i$ increase	*nd*	86	130	132
m4	*$[Ca^{2+}]i$ increase*	*nd*	3	10	10
m5	*PI hydrolysis*	24±6 (3)	4±2 (5) .	28	79
	AA release	34±9 (6)	4±2 (8)	*nd*	*nd*
	$[Ca^{2+}]i$ increase	*nd*	32	78	80

[a] Results obtained with CHO cells transfected with rat m1 and m3 $AChR_r$ respectively. All agonists were tested at 1 mM. The assays were routinely carried out in triplicates which varied by less than 10%. Data presented in Table 1 are from representative experiments (in parenthesis number of separate experiments). * McN-A-343 is used herein as a prototype M1 selective agonist (for example see Ref. 4a).
PI = increase in phosphoinositides hydrolysis; AA = increase in arachidonic acid release; cAMP = increase (for m1 and m3 AChRs) or decrease (for m2 AChR) in forskolin-stimulated increase of cAMP accumulation; $[Ca^{2+}]_i$ = increase in intracellular calcium ions concentration ; *nd* = not determined.

The structure of **AF102B** embodies the muscarinic pharmacophore in a framework of high rigidity being closely related to the rigid analog *cis*-AF30, to the semirigid muscarinic agonist acceclidine and to ACh. However, unlike **AF102B** the active diastereomer of AF30 is somewhat less M1 selective[4a,10]. We investigated in this study whether variation in selectivity can result from differences in the pharmacophoric pattern of these and some other published muscarinic agonists. This was done using a slightly modified pharmacophoric model for muscarinic activity proposed by Schulman and Sabio[11]. In this model the cationic head, the ether oxygen and the terminal methyl group were assumed to be essential for interaction with mAChRs. The question of subtype selectivity in the proposed pharmacophore was not addressed in construction of the model. Application of the model to a variety of muscarinic agonists, regardless of their selectivity, shows that satisfactory parameters for all cases could be obtained (see Table 2). Thus it appears that the muscarinic pharmacophore (as defined here) is the common denominator of the structural requirements for agonistic activity. However, in the case of pilocarpine and some of its analogs (e.g. SDZ ENS 163[17a]), the validity of such a generalized pharmacophore has been questioned[17].

In Table 2, a large spatial variability for the other heteroatom (or its equivalent) is observed for the evaluated compounds. The common pharmacophoric pattern, shared by these agonists, suggests that additional interactions might be involved in dictating selectivity. Yet no obvious correlation between the model parameters and selectivity can be found. Albeit, there seems to be a direct correlation between potency and P-O distance, neither the P-O distance nor the P-O-C-Het angle correspond to the M1/M2 relation. Therefore, selectivity in an agonist appears to depend on a more complex summation and balance of parameters including, *inter alia*: conformational rigidity, potency, the nature of Het, its spatial location, the m1-m5 mAChRs subtypes and their differential coupling with a repertoire of G-proteins in various tissues. Consequently, most of the known putative M1 selective agonists show partial activities, at least in some of the standard assays. On the other hand, full muscarinic agonists appear to be M2 selective (for highly potent M2 agonists see Ref 14), perhaps due to a more efficient coupling of M2 AChRs to effector systems[4d].

Table 2. Molecular modeling of some muscarinic agonists



Agonist	Selectivity*	P-O(Å)	P-C(Å)	O-C (Å)	P-O-C-Het (°)	Ref.
ACh	M2 > M1	6.40	8.44	2.40	-86	3c,12
Dioxolane	M2 > M1	6.51	8.71	2.42	-85	13
Muscarine	M2 > M1	6.50	8.53	2.44	-75	13
L-670,207	M2 > M1	6.75	8.79	2.47	-78	14
Methylfurmeth.	M1 > M2	6.40	8.54	2.43	-59	12
Oxathiolane	M1 > M2	6.55	8.90	2.43	-25	13
cis-AF30	M2 ≥ M1	5.90	8.15	2.47	-172	4a, 10
SND 210086	M2 ≥ M1	5.80	7.91	2.45	-120	15
AF102B	M1 > M2	5.93	8.24	2.45	-174	3,4,6
AF150	M1 > M2	5.78	7.91	2.45	-118	18
AF151	M1 > M2	5.82	8.20	2.45	-170	18
YM796S	M1 > M2	5.87	7.96	2.44	-126	16
YM796R	M1 > M2	5.81	8.06	2.43	-123	16
RS86	M1 ≥ M2	5.78	8.26	2.70	-103	15

Note: Point P corresponds to the anionic site as defined in Schulman's model[11]. Distances between the pharmacophoric elements were used instead the original P, Q parameters. All compounds were superimposed with respect to points P,O,C resulting rms < 0.3A. Dihedral angle P-O-C-Het is used to demonstrate the varying position of heteroatom or its equivalent (Het) for the different compounds. The modelling study, which included structural optimizations by means of molecular mechanics, structural comparisons and visual inspections, was performed on IRIS 4D graphic workstation. * not defined uniformly by the same method and in the same lab.

According to the presently accepted models of receptor activation and signal transduction, the efficiency of coupling between the agonist/receptor complex and the cellular repertoire of G proteins will determine the magnitude of the signal transmitted. Such coupling depends on the structure of the complex (other cellular environment being constant), and for complexes of a receptor with different agonists it will depend upon the agonist structure and its molecular properties. It follows that while different mAChR subtypes may have similar binding parameters for a given agonist, the resulting receptor/agonist complexes can display differing reactivities towards various G-proteins. That is, the structural heterogeneity of mAChRs can be manifested during the conformational changes taking place upon binding of an agonist. In the case of a flexible agonist, mutual conformational changes can occur in both receptor and ligand, yet such conformational freedom is prohibited in a rigid structure. Therefore, this is apparently the event that can be most influenced by rigidity in a given agonist, inasmuch as rigid agonists can impose only certain conformational changes in the receptor structure. Thus only distinct G-proteins are in fact activated by such agonists, followed by activation of the G-protein GTPase, (e.g. can form agonist/receptor/G-protein complexes), while others are apparently "silent". Notably, for the m1 AChR and in case of compounds like **AF102B** and two new rigid agonists **AF150** and **AF151** [18] (Fig 1, Table 1), this feature is evidenced by a selective increase in intracellular Ca^{2+}, activation of PI hydrolysis and arachidonic acid release, yet lack of significant effect on cAMP accumulation (Table 1)[9]. It is plausible, however, that the cAMP signaling pathway via m1 AChRs may exhibit less "spare receptors" compared with the other signals; this may explain the higher carbachol concentrations required for stimulating cAMP accumulation compared with other signals[9b].

Finally, rigidity in an agonist can lead to discovery of selective m1 agonists both at the level of the receptors, as well as along distinct signal transduction pathways. In this context, the highly rigid m1 agonist **AF102B**, unlike carbachol, had no effect on either cAMP levels in intact m1AChR-transfected cells, or adenylyl cyclase activity in isolated membranes prepared from the same cells[9]. **AF102B** improved memory and learning deficits in a variety of animal models, which mimic cholinergic deficits reported in AD, without producing adverse central or peripheral side-effects at the effective doses and showing a wide safety margin[3a,4,7]. This compound is a promising candidate drug and is presently in Phase II clinical trials in AD patients. Notably, mRNA for both m1AChR[19a] and Gs[19b] were markedly elevated in post-mortem brain tissues of AD patients, and an elevation of Gs and decrease of Gi proteins were reported in aged human brains[19c]. Taken together, these observations may imply increased sensitivity of m1AChR-mediated adenylyl cyclase in these situations. Albeit speculative, it is thus possible that the desired M1 or m1-selective agonists for the treatment of AD, should not stimulate adenylyl cyclase via m1AChR. Cholinergic replacement is currently being evaluated for the treatment of AD[3]. In this context both selectivity, at the level of various receptor subtypes, as well as at the level of signal transduction for the same mAChR subtype should be considered in rational drug design.

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