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European Journal of Pharmacology 355 (1998) 267–274

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Antagonist binding profile of the split chimeric muscarinic m2-trunc/m3-tail receptor

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Received 12 February 1998; revised 22 June 1998; accepted 26 June 1998

Abstract

Recent evidence suggests that G-protein-coupled receptors can behave as multiple subunit receptors, and can be split into parts, maintaining their binding ability. Transfection of a truncated muscarinic m2 receptor (containing transmembrane domains I–V, named m2-trunc) with a gene fragment coding for the carboxyl-terminal receptor portion of the muscarinic m3 receptor (containing transmembrane domains VI and VII, named m3-tail) results in the formation of a binding site with a high affinity for the muscarinic ligand *N*-[^{3}H]methylscopolamine. In this paper we analyse the antagonist binding profile of this chimeric m2-trunc/m3-tail receptor in comparison with the wild-type muscarinic m2 and m3 receptors. While many of the substances tested had an intermediate affinity for the chimeric m2-trunc/m3-tail receptor compared with m2 and m3, some compounds were able to distinguish between the chimeric m2-trunc/m3-tail receptor on the one hand and the m2 or the m3 receptor on the other. Among them, tripitramine (a high-affinity M_2 receptor antagonist) bound to the m2-trunc/m3-tail receptor with the same affinity as m2, but it bound to the m3 receptor with a 103-fold lower affinity; pirenzepine (a selective muscarinic M_1 receptor antagonist) bound to the chimeric receptor with an affinity that was 12- and 3-fold higher than that of m2 and m3, respectively. The results of this study demonstrate that the chimeric m2-trunc/m3-tail receptor has a pharmacological profile distinct from that of the originating muscarinic m2 and m3 receptors.

Keywords: Tripitramine; Pirenzepine; Muscarinic receptor subtype; Chimeric receptor; COS-7 cell

1. Introduction

Molecular cloning, sequencing, and expression studies have demonstrated the existence of five distinct muscarinic receptor subtypes (Peralta et al., 1987; Bonner et al., 1987, 1988; Kubo et al., 1988) which show a high degree of sequence homology but differ in their ligand binding and functional properties. They belong to the G-protein coupled receptor family and are predicted to be composed of seven hydrophobic transmembrane domains connected by alternating cytoplasmic and extracellular loops, an extracellular amino-terminal domain and an intracellular carboxyl-terminal segment.

Previous studies with bacteriorhodopsin, a light-driven proton pump, demonstrated for the first time that this protein can be functionally reconstituted from individual receptor fragments resulting from proteolytic cleavage of various loop regions (for a review, see Popot and Engelman, 1990). Following these pioneer experiments, similar findings have been described for G-protein coupled receptors. Kobilka et al. (1988) split the β_2-adrenoceptor into two fragments, one containing transmembrane domains I to V, and the other containing transmembrane domains VI and VII. The transfection of these two fragments together resulted in the recovery of the binding activity and function of the β_2-adrenoceptor. Since then, this phenomenon has been demonstrated for other G-protein coupled receptors: muscarinic (Maggio et al., 1993; Schöneberg et al., 1995), vasopressin (Schöneberg et al., 1996) and dopamine receptors (Barbier et al., 1996).

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0014-2999/98/$19.00
PII: S0014-2999(98)00485-3

The association of receptor domains may occur not only between fragments originating from the same receptor but also between fragments originating from different receptors. Maggio et al. (1993) demonstrated that when a truncated m2 receptor (containing transmembrane domains I–V) was co-expressed in COS-7 cells with a gene fragment coding for the carboxyl-terminal receptor portion of m3 (containing transmembrane domains VI and VII), binding sites with a high affinity for muscarinic ligands were obtained. In this study, we carried out a characterisation of the binding site of this fragmented chimeric receptor in comparison with the two wild-type muscarinic m2 and m3 receptors. We investigated both well-known compounds and newly synthesized substances. The results showed that the pharmacological profile of the fragmented chimeric m2-trunc/m3-tail receptor is different from that of the wild-type muscarinic m2 and m3 receptors.

2. Materials and methods

2.1. Receptor constructs

Hm2pcD and Rm3pcD, two mammalian expression vectors containing the entire coding sequence of the human muscarinic m2 and the rat muscarinic m3 receptors, were used to construct the fragmented chimeric (m2-trunc/m3-tail) receptor gene. The pcDm2-trunc gene was constructed by removing a 5.3-kb *Sst*I-fragment from Hm2pcD and self-ligating the remaining receptor, this resulted in the creation of a stop codon after the amino acid codon Ser-283 of the human m2 sequence. For the creation of pcDm3-tail, a 1.3-kb *Bam*HI–*Pst*I restriction fragment was cut out from Rm3pcD and replaced with a synthetic DNA linker sequence containing an in-frame translation initiation codon (ATG) and a short stretch of adjacent 5′ untranslated sequence of Rm3pcD. The resulting construct coded for the 202 C-terminal amino acids (from Leu-388 to Leu-589) of the rat muscarinic m3 receptor. A plasmid containing the two transcriptional units (m2-trunc and m3-tail) was constructed by transposing a blunt-ended *Sal*I-*Tth*IIII fragment containing the SV40 early region promoter, the m3-tail codon sequence and the segment carrying the SV40 late region polyadenylation signal from pcDm3-tail into the blunt-ended *Sal*I site present in pcDm2-trunc. More details will be found in Maggio et al. (1993).

2.2. Cell culture and transfection procedure

Kidney SV40 transformed, Africa green monkey (COS-7) cells were incubated at 37°C in a humidified atmosphere (5% CO_2) and grown in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal bovine serum, 2% (vol/vol) L-glutamine 200 mM, 1% (vol/vol) penicillin (10 000 units/ml) and streptomycin (10 mg/ml) solution, and 1% (vol/vol) minimal essential medium non-essential amino acid solution. All these products were purchased from Sigma (St. Louis, MO, USA) and tissue culture supplies were from Falcon (Becton Dickinson Labware, Bedford, MA, USA). The cells were seeded at a density of $\sim 1.5 \times 10^6$ cells per 100-mm dish and the day after transiently transfected with the plasmid DNA (4 μg per dish) by a DEAE-dextran method (Cullen, 1987).

2.3. Membrane preparation and binding assay

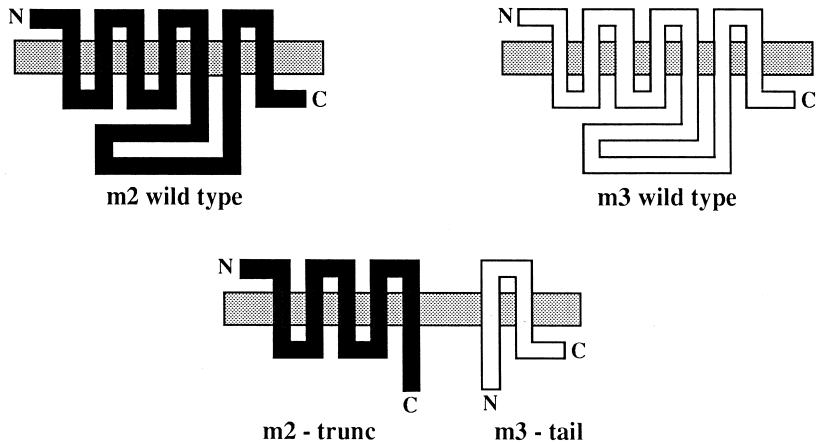
Three days after transfection, cells were washed twice with phosphate-buffered saline, scraped from the 100-mm plates into ice-cold binding buffer (25 mM sodium phosphate containing 5 mM magnesium chloride at pH 7.3) and homogenized for 30 s using a Polytron (setting 5). Membranes were pelleted at $18{,}000 \times g$ for 30 min at 4°C. The pellet was resuspended in 5 ml ice-cold binding buffer and the membranes were rehomogenized. Saturation experiments were performed with seven *N*-[^{3}H]methylscopolamine concentrations (12.5–800 pM). Inhibition experiments were carried out with 8 different concentrations of the cold antagonist, against an *N*-[^{3}H]methylscopolamine concentration of 200 pM. Atropine (1 μM) was used to define non-specific binding. Incubation was at room temperature for 3 h. The bound ligand was separated on glass fiber filters (Whatman, GF/B) with a Brandel Cell Harvester. The filters were washed three times with 4 ml ice-cold binding buffer and transferred to vials, 4 ml of scintillation liquid was added, and counts were performed with a β-counter. Protein dosage was performed using the method described by Lowry et al. (1951).

2.4. Statistical evaluation

Data are presented as mean values ± S.E.M of at least two separate experiments, each performed in triplicate. Affinity constants (K_d and K_i), B_{max} and Hill coefficients were derived using least squares regression analyses of the Kaleidagraph software running on a Macintosh computer. Deviation of the Hill coefficients from unity was assessed using Student's one-tailed *t*-test for unpaired data; $P < 0.05$ was accepted as being significant.

2.5. Ligands

N-[^{3}H]methylscopolamine (84 Ci/mmol) was purchased from Amersham (UK). Atropine and pirenzepine were from Sigma. 4-DAMP (4-diphenylacetoxy-*N*-methylpiperidine) and *p*-F-HHSiD (*para*-fluoro-hexahydrosiladifenidol) were obtained from Research Biochemicals



	TRANSMEMBRANE DOMAIN VI	TRANSMEMBRANE DOMAIN VII
Human m2	T I L A I L L A F I I T W A P Y N V M V L I N T	N T V W T I G Y W L C Y I N S T I N P A C Y A
Rat m3	T L S A I L L A F I I T W T P Y N I M V L V N T	K T Y W N L G Y W L C Y I N S T V N P V C Y A
	↑ ↑ ↑ ↑ ↑	↑ ↑ ↑ ↑ ↑ ↑

Fig. 1. Schematic representation of the wild-type m2 and m3, and the chimeric muscarinic m2-trunc/m3-tail receptors. The wild-type m2 and m3 receptors are composed of seven hydrophobic domains crossing the plasma membrane (shaded area) connected by alternating cytoplasmic and extracellular loops, an extracellular amino-terminal domain and an intracellular carboxyl-terminal segment. The m2-trunc fragment contains the amino-terminal domain, the first five hydrophobic transmembrane regions and the initial portion (56 amino acids) of the third cytoplasmic loop of the wild-type m2 receptor. The m3-tail fragment contains the final portion (105 amino acids) of the third cytoplasmic loop, the last two hydrophobic transmembrane domains and the carboxyl-terminal segment of the wild-type muscarinic m3 receptor. The amino acid differences in the transmembrane domains VI and VII of the human muscarinic m2 and the rat muscarinic m3 receptors are presented.

(Natick, MA, USA). The enantiomer (1*S*,2*S*)*S*-1 of rociverine (Barbier et al., 1995) was from Laboratori Guidotti (Pisa, Italy). [SS 62, CH_3I] and [SS 63, CH_3I] were a gift of Prof. Fulvio Gualtieri. All other substances were synthesized in the Department of Pharmaceutical Sciences, University of Bologna.

Table 1
Binding affinities of several compounds for the chimeric m2-trunc/m3-tail receptor compared with the wild-type m2 and m3 muscarinic receptors

Compound	m2 (K_i, nM)	m2-trunc/m3-tail (K_i, nM)	m3 (K_i, nM)
[^{3}H]NMS (K_d)	0.22 ± 0.02	0.10 ± 0.01	0.08 ± 0.01
4-DAMP	3.41 ± 0.50	1.23 ± 0.11	0.42 ± 0.03
p-F-HHSiD	231 ± 20.8	86.3 ± 5.33	19.4 ± 1.93
Methoctramine	14.3 ± 1.46	52.7 ± 3.19	207 ± 11.9
Tripitramine	0.26 ± 0.02	0.34 ± 0.02	35.1 ± 3.32
CC 8	1.02 ± 0.07	1.49 ± 0.09	32.4 ± 2.81
CC 9	11.8 ± 1.31	30.9 ± 4.29	581 ± 36.6
Dipitramine	1.21 ± 0.16	3.43 ± 0.18	58.0 ± 6.23
ML116	0.19 ± 0.01	0.36 ± 0.04	2.24 ± 0.41
ML105	10.4 ± 0.93	13.4 ± 1.05	26.5 ± 3.56
ML187	137 ± 7.30	297 ± 19.8	1071 ± 97.5*
ML121	2.00 ± 0.17	2.91 ± 0.31	1.92 ± 0.20
AO 47	4.69 ± 0.72	14.2 ± 3.21	91.2 ± 8.25
ML183	278 ± 32.6	687 ± 52.0	230 ± 19.6
CC 21	57.1 ± 6.71	566 ± 43*	195 ± 13.1
Pirenzepine	491 ± 51.4	38.9 ± 5.48	108 ± 6.54
Rociverine (μM)	135 ± 15.3	52.7 ± 6.30	27.3 ± 4.88
SS 62, CH_3I	150 ± 12.9	148 ± 15.4	198 ± 21.0
SS 63, CH_3I	60.9 ± 8.32	40.6 ± 3.65	12.2 ± 1.07

The affinity estimates were derived from *N*-[^{3}H]methylscopolamine displacement experiments and represent the mean ± S.E.M. Hill coefficients were omitted because for all the compounds tested, except for the two indicated by the asterisk, they were not different from unity.

3. Results

As has been previously demonstrated, fragments originating from the m2 and the m3 receptors (Fig. 1) are able to interact (Maggio et al., 1993); when a truncated m2 receptor (containing transmembrane domains I–V, named m2-trunc) is co-expressed in COS-7 cells with a gene fragment coding for the carboxyl-terminal receptor portion of m3 (containing transmembrane domains VI and VII, named m3-tail), binding sites with a high affinity for the muscarinic ligand *N*-[^{3}H]methylscopolamine are obtained (Table 1). In order to define the pharmacological profile of this chimeric m2-trunc/m3-tail receptor we tested a series of muscarinic antagonists.

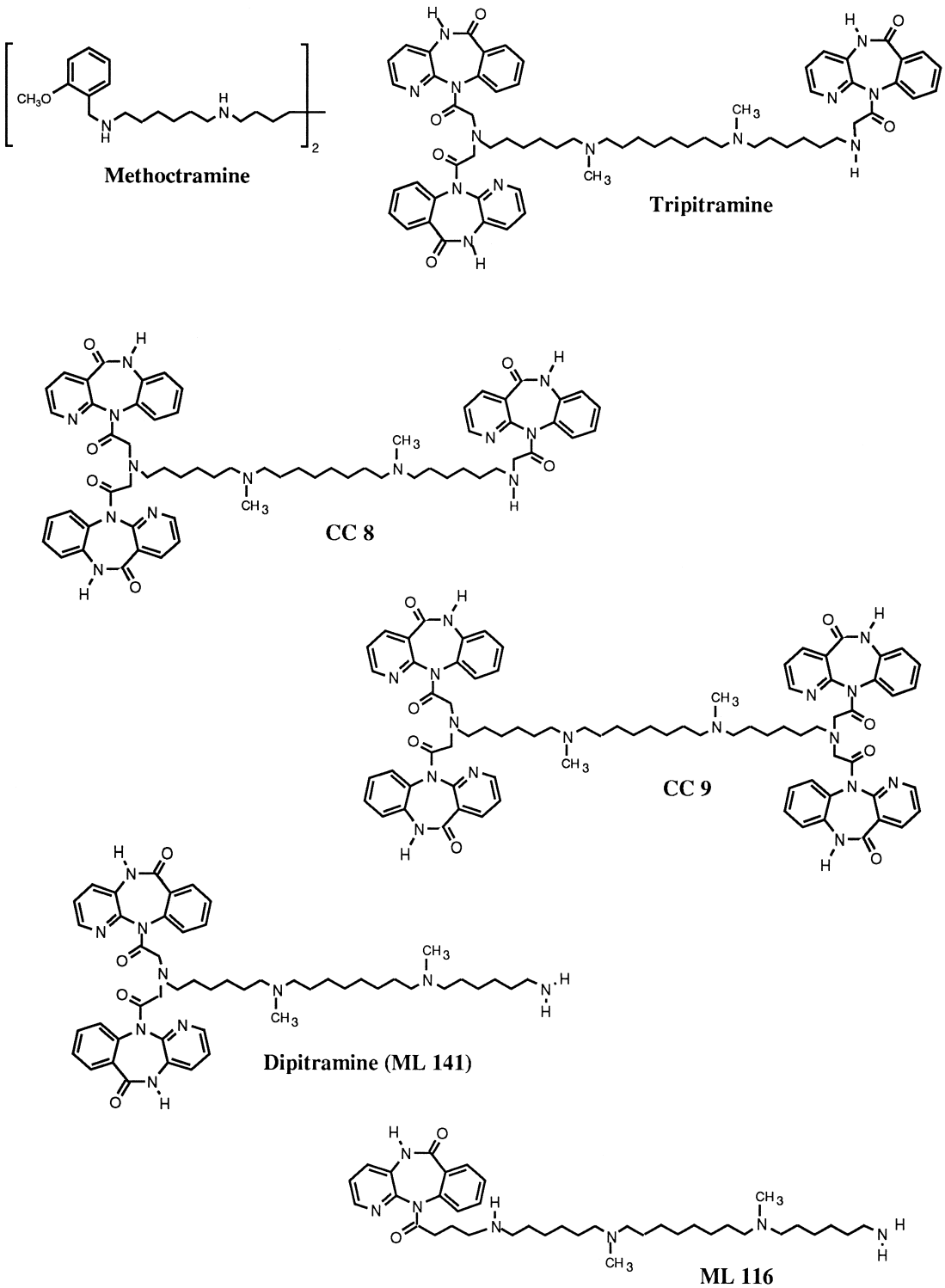
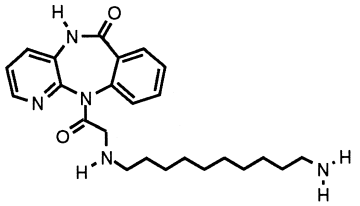
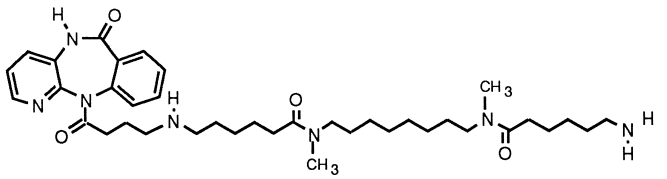


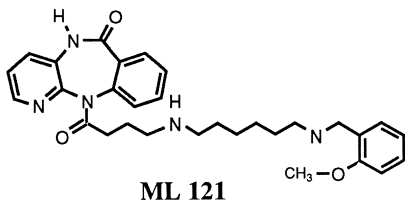
Fig. 2. Chemical structures.



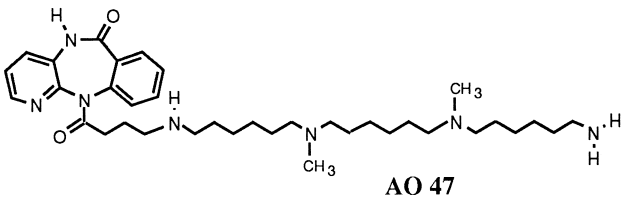
ML 105



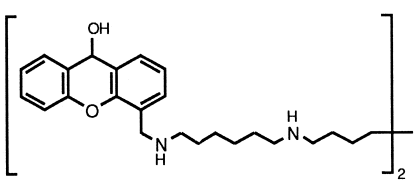
ML 187



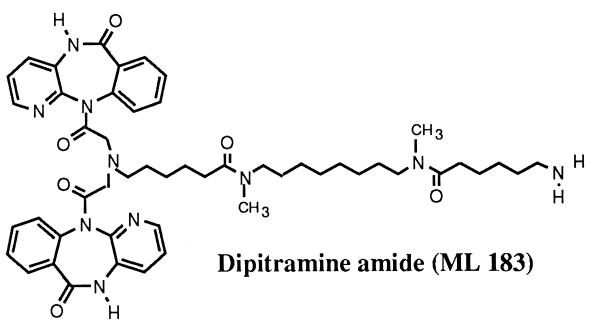
ML 121



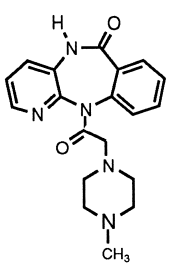
AO 47



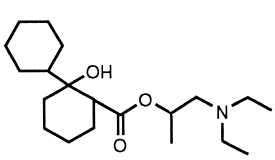
CC 21



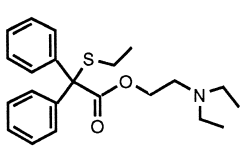
Dipitramine amide (ML 183)



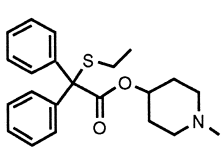
Pirenzepine



Rociverine



SS 62



SS 63

Fig. 2 (continued).

Our pharmacological characterization started with the two high-affinity M_3 antagonists 4-DAMP and pF-HHSiD. As is shown in Table 1, both of them showed an intermediate affinity for the chimeric m2-trunc/m3-tail receptor compared with m2 and m3 (the affinity profile was m3 > m2-trunc/m3-tail > m2). Similar results were obtained with the selective m2 antagonist methoctramine which shows an inverse affinity profile: m2 > m2-trunc/m3-tail > m3.

Tripitramine (Fig. 2), a new selective high-affinity M_2 antagonist, unexpectedly showed an affinity for the chimeric receptor that was not different from that of the wild-type m2 receptor. As the affinity of tripitramine for m3 compared with m2-trunc/m3-tail is 103-fold lower,

this compound can readily differentiate between these two receptors. A series of tripitramine analogues (Fig. 2) were tested to better characterize the binding requirement of the chimeric m2-trunc/m3-tail receptor. Dipitramine lacks an 11-acetyl-5,11-dihydro-benzole[*e*]pyrido[3,2-*b*][1,4]diazepin-6-one (PBD) group compared with tripitramine; this resulted in a 10-fold reduction in binding affinity, while conversely the selectivity with respect to m3 was maintained. The substitution of all the PBDs of tripitramine with 11-acetyl-6,11-dihydro-benzo[*b*]pyrido[2,3-*e*][1,4]diazepin-5-one moieties (inPBDs), as in CC8, had a more limited effect on the affinity (4-fold); the difference in affinity with respect to m3 was again maintained. A great loss in affinity with respect to tripitramine (91-fold) was shown by the analogue CC9, which has 4 inPBDs bound to the molecule; still this molecule maintains a high affinity ratio between m2-trunc/m3-tail and m3. This difference in affinity between the chimeric receptor and m3 was greatly reduced by the compounds with only one PBD (ML116, ML105, ML187, ML121 and AO47) (Fig. 2): none of these compounds showed more than 6-fold higher affinity for the chimeric m2-trunc/m3-tail receptor with respect to m3 (Table 1). ML116 has the same affinity as tripitramine for the chimeric m2-trunc/m3-tail receptor and for m2. Remarkably, ML187, in which two amine functions were converted into amide groups, lost more than 100-fold affinity for m2 and m2-trunc/m3-tail compared with ML116. The same modification induced a comparable loss in affinity for m2 and m2-trunc/m3-tail in ML183 with respect to dipitramine.

CC21, a compound closely related to methoctramine (Fig. 2) showed a low affinity for the chimeric m2-trunc/m3-tail receptor, whereas the loss in affinity for m2 (compared with methoctramine) was only 4-fold.

Pirenzepine is a very well-known selective M_1 receptor antagonist; in our study, we found that it has a higher affinity for the chimeric receptor compared with m3 (3-fold) and m2 (13-fold). Rociverine, another M_1 preferring (low-affinity) antagonist (Barbier et al., 1995), did not show the same affinity profile as pirenzepine; its affinity for the chimeric m2-trunc/m3-tail receptor was intermediate between those of the m2 and the m3 receptors.

The last two compounds that we tested were [SS 62, CH_3I] and [SS 63, CH_3I] (Fig. 2). These compounds have been shown to be potent antagonists in functional assays (see Section 4). In our study, they did not prove to have a particular preference for the chimeric m2-trunc/m3-tail receptor, and their affinities for the chimera were intermediate between those of m2 and m3.

4. Discussion

The results of this study clearly demonstrate that the chimeric m2-trunc/m3-tail receptor exhibits the binding characteristics of a classic muscarinic receptor; nevertheless, its pharmacological profile differs significantly from that of the generating wild-type muscarinic m2 and m3 receptors.

As has been shown in a previous study (Maggio et al., 1993) the chimeric m2-trunc/m3-tail receptor binds the physiological neurotransmitter acetylcholine and the agonist carbachol with an affinity which is intermediate between those found for the two generating m2 and m3 receptors, indicating that the binding pocket created by the structural assembly of the two receptor fragments retains the conformation of muscarinic receptors. In agreement with this findings, the affinity of the non-specific muscarinic antagonist *N*-[^{3}H]methylscopolamine for the chimeric m2-trunc/m3-tail was in the order of magnitude found for all the other muscarinic receptors.

While it is predictable that non-specific muscarinic antagonists will not distinguish the chimeric receptor from the wild-type m2 and m3 receptors, selective antagonists should probably recognize the structural differences. For this reason, we started our pharmacological analysis with two selective muscarinic M_3 receptor antagonists: 4-DAMP and *p*-F-HHSiD. These compounds showed respectively an 8- and 12-fold higher affinity for m3 with respect to m2 and an intermediate affinity for the chimeric receptor. If we consider that five of the transmembrane domains that constitute the chimeric receptor derive from the m2 receptor, we can assume that the carboxyl-terminal domain of m3 increases the affinity for 4-DAMP and *p*-F-HHSiD. It is interesting to note that similar results were obtained by Wess et al. (1990) with the chimeric m2N3 receptor using 4-DAMP and HHSiD (a non-fluorinated analogue of *p*-F-HHSiD). In their study Wess et al. (1990) assert that 'the subtype-selective binding of muscarinic antagonists is critically, although not exclusively, dependent on the carboxyl-terminal receptor domain'. The m2N3 chimera has been constructed by joining the m2 and m3 receptors roughly in the middle of the transmembrane domain VI. As the part of the transmembrane domain VI that belongs to m2 is identical to that of m3, the only difference between the chimeric m2N3 receptor and our fragmented chimeric m2-trunc/m3-tail receptor is the terminal part of the third cytoplasmic loop, which in the case of m2N3 belongs to m2, while in the case of m2-trunc/m3-tail, it belongs to m3. Assuming that the hydrophilic N-terminal receptor portion of the third cytoplasmic loop is not involved in antagonist binding, we could consider these two receptors identical as far as the amino acid sequence involved in antagonist binding is concerned.

Methoctramine is a well-known muscarinic M_2 selective antagonist which in our experiments showed a 15-fold higher affinity for m2 compared with m3. The affinity found for the chimeric m2-trunc/m3-tail receptor was intermediate; again similar findings were shown in the paper of Wess et al. (1990), supporting their conclusion of the importance of the carboxyl-terminal receptor domain in antagonist binding.

A recently synthesized muscarinic M_2 receptor ligand, tripitramine, has shown a higher selectivity for m2 compared with methoctramine (Maggio et al., 1994). Tripitramine has shown a 135-fold higher affinity for m2 with respect to m3, while surprisingly, the chimeric m2-trunc/m3-tail receptor binds tripitramine as well as m2. In this case, it seems that the carboxyl-terminal segment of m3 has not affected the binding of the antagonist tripitramine. In order to better understand how this compound binds to the chimeric receptor, we tested a series of tripitramine analogues. CC8 is a tripitramine analogue in which the PBDs have been replaced by inPBDs; this compound maintains the same affinity ratio between m2 and m2-trunc/m3-tail receptors, but it is 4-fold less potent compared with tripitramine. The addition to CC8 of a fourth inPBD to form CC9 results in a drastic loss of affinity for all three receptors, indicating that the steric hindrance of the added group is unfavourably accepted by the muscarinic binding pocket. Subtraction of a PBD from tripitramine to form dipitramine decreases the affinity for m2 and m2-trunc/m3-tail by 4 and 10 times, respectively, indicating that this group actively interacts with the binding sites of these two receptors to increase the affinity. It is important to notice that in order to maintain an affinity ratio of more than one order of magnitude between m3 on the one hand, and m2-trunc/m3-tail on the other, it is important to have at least two PBDs bound to the molecule. Consequently, the selectivity of ML116, ML105, ML187, ML121 and AO47, which have only one PBD, is considerably reduced.

Modification of the polymethylene chain variably affects the binding; for example ML116, which has a butyryl instead of an acetyl moiety between the tricyclic system and the terminal amino group, maintains a high affinity for both m2 and m2-trunc/m3-tail, while the reduction of the distance between the two central amino groups from 8 to 6 carbons, as in AO47, reduces the affinity for m2 and m2-trunc/m3-tail. A more pronounced decrease in affinity is obtained by the transformation of two amine functions into two amide groups (compare ML187 with ML116, and ML183 with dipitramine). Truncation of the polymethylene tetraamine chain and addition of a 2-methoxybenzyl group, as in ML121, also reduce the affinity for m2 and m2-trunc/m3-tail. All these tripitramine analogues showed a difference in affinity between m2 and m2-trunc/m3-tail of not more than 3-fold, suggesting that the PBDs probably recognize either on m2 and on the chimeric m2-trunc/m3-tail identical amino acid determinants in the binding pockets. This is explained by the high homology existing between the two receptors. If we exclude the C-terminal portion of the third cytoplasmic loop (probably not involved in antagonist binding) only a few amino acids in the transmembrane domains VI and VII differentiate m2 from m2-trunc/m3-tail (Fig. 1).

The structure of CC21 is closely related to that of methoctramine, rather than tripitramine, as confirmed also by the fact that CC21 was only 4-fold less potent than, and equipotent to, methoctramine at m2 and m3 receptors, respectively. From a chemical point of view, methoctramine and CC21 structures can easily be superimposed, the only difference between them being part of the tricyclic moiety of CC21, namely a phenyl ring and the hydroxy function, which is lacking in methoctramine. It derives that the significant loss of affinity with respect to methoctramine displayed by CC21 towards the m2-trunc/m3-tail receptor can probably be ascribed to the tricyclic moiety of CC21, which may interact with the chimeric receptor differently compared with m2 and m3 receptors. This reasoning is in agreement with the observation that the Hill coefficient for CC21 was significantly different from unity for the chimeric receptor but not for m2 and m3 receptors.

To continue our pharmacological characterization, we tested two muscarinic M_1 receptor antagonists: pirenzepine, a well-known high-affinity ligand, and rociverine (the enantiomer (1*S*,2*S*)*S*-1, see Barbier et al., 1995), a low-affinity compound that we have recently characterized on CHO-K1 cell lines stably transfected with the five muscarinic receptors. We selected rociverine because it has a pattern of selectivity for the muscarinic receptors very similar to pirenzepine.

While rociverine showed a similar affinity for the three receptors tested, pirenzepine was the only substance in our study that showed a higher affinity for the chimeric m2-trunc/m3-tail receptor compared with m2 and m3; the affinity ratios of m2-trunc/m3-tail vs. m2 and m3 were 12- and 3-fold, respectively. This result was quite unexpected, considering the fact that pirenzepine, like tripitramine and many of its congeners mentioned above, contains a PBD group. In this case, it is clear that the piperazine ring of pirenzepine determines the specificity for the chimeric m2-trunc/m3-tail receptor.

The last two compounds that we tested have been shown to differentiate clearly between guinea pig atrium and guinea pig ileum when tested in functional experiments (Angeli et al., 1993), but when they were tested on cell lines stably transfected with muscarinic receptors, they completely lost their selectivity. One possible explanation for this discrepancy could be that in tissues, the presence of more than one muscarinic receptor subtype could lead to the formation of chimeric receptors with a higher affinity for these antagonists. This conviction was sustained by the fact that muscarinic m2 and m3 subtypes coexist in smooth muscle. Our expectation was disappointed because the affinity we found for our chimeric m2-trunc/m3-tail receptor was intermediate between those found for m2 and m3.

In conclusion, our results demonstrate that the chimeric m2-trunc/m3-tail receptor has a peculiar pharmacological profile. Our screening has identified substances able to discriminate between the chimeric m2-trunc/m3-tail receptor on the one hand and the muscarinic m2 or m3

receptors on the other. Among the compounds investigated, pirenzepine and CC21 emerge as possible lead compounds to design selective antagonists for the m2-trunc/m3-tail receptor vs. m2 and m3 receptors, or for m2 and m3 receptors vs. the chimeric receptor, respectively. In future studies we will utilize these compounds to see whether the contemporaneous presence of the two muscarinic m2 and m3 receptor subtypes in the same cells leads to the formation of the chimeric m2-trunc/m3-tail receptor.

Acknowledgements

The authors would like to thank Prof. R. Packham for his help in restyling the English of the manuscript.

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