the minimum effective antagonist pharmacophore might consist of a basic group attached to the cyclic hexapeptide ring. We therefore questioned the role of proline at position 7 in the antagonist. Proline is proposed to play a key role in the *agonist* pharmacophore both as an important binding element and in its ability to properly orient the tripeptide tail with respect to the cyclic hexapeptide ring.[2] In an attempt to evaluate the importance of proline at position 7, we prepared compounds **6** and **7** in which this group is deleted.

Compound **6** was prepared by solid-phase synthesis on benzhydrylamine resin, cleaved with anhydrous liquid HF, and cyclized with dilute aqueous potassium ferricyanide. This peptide was purified by countercurrent distribution followed by gel filtration. Compound **7** was prepared in an analogous way to compound **4** with the cyclic hexapeptide acid **8** as a key intermediate. Acid **8** was prepared by solid-phase synthesis on Merrifield resin, cleaved, and oxidized in the same manner as **5**.

The biological activities of **6** and **7** are given in Table I. Both compounds retain very good in vitro activity compared to that of **3**; however, their in vivo activities are somewhat reduced. This result may reflect metabolic as well as pharmacokinetic differences. It is clear from these results that both a terminal carboxamide and the proline residue are not essential for potent antagonist activity; a basic moiety attached directly to the cyclic hexapeptide ring presents an effective pharmacophore. These observations help to further refine our understanding of the molecular interactions of peptide antagonists with the vasopressin V_2-receptor.

Acknowledgment. We thank Gerald Roberts and Susan Rottschaefer for obtaining the FAB mass spectra. We are also indebted to Maurice Manning (Medical College of Ohio at Toledo) for providing a sample of antagonist **1**.

Registry No. **1**, 80148-24-9; **2**, 96827-97-3; **3**, 98612-53-4; **4**, 98612-54-5; **5**, 98612-55-6; **6**, 98612-56-7; **7**, 98612-57-8; **8**, 98612-58-9; mono-Boc-cadaverine, 51644-96-3; adenylate cyclase, 9012-42-4.

† Department of Peptide Chemistry.
‡ Department of Pharmacology.
§ Department of Molecular Pharmacology.

**William F. Huffman,*† Fadia E. Ali,† William M. Bryan†
James F. Callahan,† Michael L. Moore†
Joanne S. Silvestri,† Nelson C. F. Yim†
Lewis B. Kinter,‡ Jeanne E. McDonald‡
Daryl Ashton-Shue,‡ Frans L. Stassen§
Grace D. Heckman,§ Dulcie B. Schmidt,§ Lynn Sulat§**

*Departments of Peptide Chemistry, Pharmacology, and Molecular Pharmacology
Smith Kline & French Laboratories
Philadelphia, Pennsylvania 19101*

Received June 7, 1985

Articles

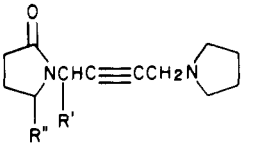
Stereoselectivity of Muscarinic Receptors in Vivo and in Vitro for Oxotremorine Analogues. *N*-[4-(Tertiary amino)-2-butynyl]-5-methyl-2-pyrrolidones

René Amstutz,† Björn Ringdahl,* Bo Karlén,‡ Margareth Roch, and Donald J. Jenden

Department of Pharmacology, School of Medicine, and Brain Research Institute, University of California, Los Angeles, California 90024. *Received November 30, 1984*

The enantiomers of three 5-methyl-2-pyrrolidone analogues of the muscarinic agent oxotremorine (**1**) were synthesized. The pyrrolidine derivative (*R*)-**13** was an antagonist to carbachol in the guinea pig ileum and also showed central and peripheral antimuscarinic activity in vivo. It was more potent and more selective than atropine in antagonizing the central effects of **1**. The dimethylamino analogue (*R*)-**14** and the trimethylammonium salt (*R*)-**15** were potent agonists in the guinea pig ileum. (*R*)-**14** showed both central muscarinic (hypothermia) and central antimuscarinic activity (antagonism of oxotremorine-induced tremor) in vivo. The *R* enantiomers of **13**–**15** were considerably more potent than the *S* enantiomers in vivo and in vitro irrespective of whether agonist or antagonist activity was measured. From a comparison of the contribution of the methyl group at the chiral center to the overall affinities, it is suggested that agonists and antagonists in this series bind in an essentially identical manner to the muscarinic receptor.

Methyl substitution in the potent muscarinic agent oxotremorine (**1**) has pronounced effects on its pharmacological activity.[1-4] Only one of the structural isomers resulting from the introduction of a single methyl group in **1** has oxotremorine-like properties in vivo.[4,5] The remaining isomers inhibit the effects of **1**.[1-4] The most potent among these are compounds **2** and **13**, which are more effective than atropine in antagonizing the central effects of **1**. In comparison to atropine, however, **2** and **13** show relatively weak peripheral parasympatholytic activity.[4-7] Practically all of the antimuscarinic activity of **2** resides in the *R* enantiomer.[8] For example, (*R*)-**2** was



1. R' = R" = H
2. R' = CH_3, R" = H
13. R' = H, R" = CH_3

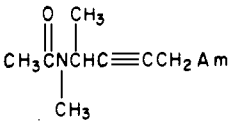
† Present address: Preclinical Research, Sandoz Ltd., CH-4002 Basel, Switzerland.
‡ Present address: KabiVitrum AB, S-11287 Stockholm, Sweden.

(1) Dahlbom, R. In "Cholinergic Mechanisms: Phylogenetic Aspects, Central and Peripheral Synapses, and Clinical Significance"; Pepeu, G., Ladinsky, H., Eds.; Plenum Press: New York, 1981; p 621.

0022-2623/85/1828-1760$01.50/0

200–250 times more potent than (*S*)-**2** in inhibiting carbachol-induced contractions of the guinea pig ileum.[4,9,10] Previous results suggested that the methyl group of (*R*)-**2** provided an additional interaction with the muscarinic receptor.[4,10] It also appeared that the methyl group of **13** participated in such an interaction.[4] If this were true, **13** also would be expected to exhibit stereoselectivity of action, especially since the 2- and 3-methylpyrrolidino analogues of **1**, in which the methyl group does not seem to provide additional binding, display little or no stereoselectivity.[4,11] We now have synthesized the enantiomers of **13** to investigate further the relationship between the binding of **1**, **2**, and **13** to muscarinic receptors.

We recently showed that in some closely related *N*-methylacetamide analogues (**3–5**) of **1** having a chiral center in position 1 of the butynyl chain the *R* isomer was more potent than the *S* isomer both in vivo and in vitro, regardless of whether the compounds were agonists, partial agonists, or antagonists.[9] Thus, the stereochemical requirements for muscarinic and antimuscarinic activity among these acetamides appeared to be identical,[9] in contrast to the different stereochemical demands of more conventional muscarinic agonists and antagonists.[12] However, no chiral agonist analogue of **1** having a 2-pyrrolidone moiety has yet been resolved. We previously found[3] that the dimethylamino analogue of **13**, i.e. **14**, was a rather potent muscarinic agonist, which provided us with an opportunity to investigate a chiral agonist closely related to **1**. We therefore prepared the enantiomers of **14** as well as of the corresponding trimethylammonium analogue (**15**). Thus, a series of structurally related enantiomeric pairs (**13–15**), all having a chiral center at the 5-position of the pyrrolidone ring, became available for further investigation of the relationship between agonist and antagonist actions at the muscarinic receptor.



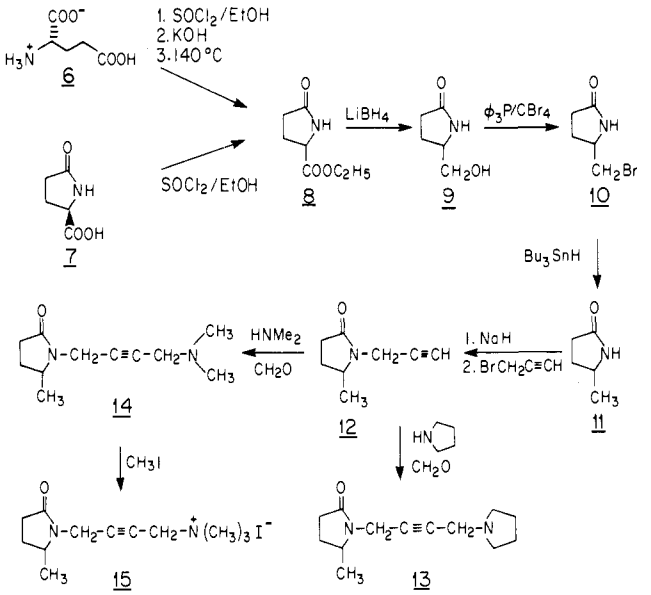
3. Am = 

4. Am = $N(CH_3)_2$

5. Am = $N^+(CH_3)_3$

Chemistry. The *R* enantiomers of **13–15** were synthesized from L-glutamic acid (**6**), which was transformed to (*S*)-5-(bromomethyl)-2-pyrrolidone [(*S*)-**10**] by using minor modifications of a previously described method[13] (Scheme I). Thus, **6** was esterified with $SOCl_2$ and EtOH, and the resulting ammonium salt was treated with KOH. Cyclization at 140 °C yielded (*S*)-5-carbethoxy-2-pyrrolidone [(*S*)-**8**]. Reduction of (*S*)-**8** with $LiBH_4$ gave (*S*)-5-(hydroxymethyl)-2-pyrrolidone [(*S*)-**9**], which was smoothly converted to (*S*)-**10** with triphenylphoshine and CBr_4.[14] (*S*)-**10** was then treated with tributyltin hydride in the presence of azoisobutyronitrile as a radical initiator to give (*R*)-5-methyl-2-pyrrolidone [(*R*)-**11**]. Deprotonation of (*R*)-**11** with NaH and alkylation with propargyl bromide[15] produced a mixture of (*R*)-5-methyl-*N*-2-propynyl-2-pyrrolidone [(*R*)-**12**] and the corresponding allene isomer in a ratio of about 6:1. The formation of allene isomer has been noted previously in the propargylation of methyl-substituted 2-pyrrolidones.[5] Substitution of BuLi for NaH increased the amount of unwanted allene isomer. Without further purification of the mixture, (*R*)-**12** was aminomethylated (Mannich reaction) to the oxotremorine analogues (*R*)-**13** and (*R*)-**14**. For the synthesis of the *S* enantiomers of **13–15**, D-pyroglutamic acid (**7**) was used as starting material in an analogous reaction sequence (Scheme I). That optical isomer of **8–15** which was synthesized from D-pyroglutamic acid consistently had lower optical rotation at the sodium D line than its enantiomer prepared from L-glutamic acid. This observation suggests that the D-pyroglutamic acid used was not optically pure. It may be assumed that the natural L-glutamic acid is optically pure and that no racemization occurs in the transformation of **6** and **7** to **13–15**. The latter assumption is reasonable as no bonds to the chiral center are broken. The *R* enantiomers of **13–15** should therefore be optically pure. The optical purities of the *S* enantiomers of **13–15** should then be 92.5–94% as calculated from their D-line rotations.

Scheme I



Pharmacological Results

Guinea Pig Ileum. The enantiomers of **13–15** were tested for muscarinic and antimuscarinic activity in the isolated guinea pig ileum. The results from these tests are summarized in Table I, which also includes relevant data for oxotremorine and atropine as well as previously published[3-5,10] results for (*R*)-**2**, (*S*)-**2**, (±)-**13** and (±)-**14**. The enantiomers of **14** and **15** elicited contractions of the guinea

(2) Resul, B.; Ringdahl, B.; Dahlbom, R. *Acta Pharm. Suec.* **1979**, *16*, 161.
(3) Ringdahl, B.; Jenden, D. J. *Life Sci.* **1983**, *32*, 2401.
(4) Ringdahl, B.; Jenden, D. J. *Mol. Pharmacol.* **1983**, *23*, 17.
(5) Ringdahl, B.; Muhi-Eldeen, Z.; Ljunggren, C.; Karlēn, B.; Resul, B.; Dahlbom, R.; Jenden, D. J. *Acta Pharm. Suec.* **1979**, *16*, 89.
(6) Lindgren, S.; Lindquist, A.; Lindeke, B.; Svensson, U.; Karlēn, B.; Dahlbom, R.; Blair, M. R., Jr. *Experientia* **1970**, *26*, 1232.
(7) Lindgren, S.; Lindquist, A.; Lindeke, B.; Svensson, U.; Karlēn, B.; Dahlbom, R.; Blair, M. R., Jr. *Acta Pharm. Suec.* **1973**, *10*, 435.
(8) Dahlbom, R.; Lindquist, A.; Lindgren, S.; Svensson U.; Ringdahl, B.; Blair, M. R., Jr. *Experientia* **1974**, *30*, 1165.
(9) Dahlbom, R.; Jenden, D. J.; Resul, B.; Ringdahl, B. *Br. J. Pharmacol.* **1982**, *76*, 299.
(10) Ringdahl, B. *J. Pharmacol. Exp. Ther.* **1984**, *229*, 199.
(11) Ringdahl, B.; Dahlbom, R. *Acta Pharm. Suec.* **1978**, *15*, 255.
(12) Triggle, D. J.; Triggle, C. R. "Chemical Pharmacology of the Synapse"; Academic Press: New York, 1976; p 233.
(13) Silverman, R. B.; Levy, M. A. *J. Org. Chem.* **1980**, *45*, 815.
(14) Appel, R. *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 801.
(15) Sterk, L.; Deak, G.; Gyorgy, L. *Acta Chim. Acad. Sci. Hung.* **1973**, *77*, 109.

Table I. Muscarinic, Antimuscarinic, and Nicotinic Effects and Acute Toxicity of Some Oxotremorine Analogues[a]

compd	guinea pig ileum			ED_{50}, μmol/kg		frog rectus abdominis	acute tox: LD_{50},
	ED_{50}, μmol/L	EPMR[b]	K_B, μmol/L	mydriatic act.[c]	tremorolytic act.[d]	EPMR[b]	μmol/kg
(S)-2			9.5 ± 0.6 (4)		20		
(R)-2			0.046 ± 0.004 (4)		0.26		
(±)-13			0.093	9.0 ± 0.53	0.4	116 ± 14 (3)	
(S)-13			0.98 ± 0.095 (4)	66 ± 25	4.2 ± 0.6	132 ± 22 (4)	40 ± 5.2
(R)-13			0.051 ± 0.008 (4)	4.8 ± 0.33	0.19 ± 0.01	141 ± 13 (4)	45 ± 5.8
(±)-14	0.55	8.8			5.5		
(S)-14	4.6 ± 0.4 (4)	73.9 ± 6.8 (4)			~50	inact	183 ± 24
(R)-14	0.29 ± 0.02 (8)	4.6 ± 0.22(8)			2.4 ± 0.1	inact	426 ± 55
(S)-15	1.3 ± 0.09 (6)	20.0 ± 1.5 (6)				8.8 ± 1.0 (4)	3.5 ± 0.5
(R)-15	0.076 ± 0.004 (6)	1.2 ± 0.06 (6)				10.0 ± 1.3 (4)	8.9 ± 1.2
atropine			0.00085 ± 0.0001 (4)	0.43 ± 0.043	0.86 ± 0.08		104 ± 14
oxotremorine (1)	0.026 ± 0.002 (6)	0.42 ± 0.03 (6)				49 ± 2.9 (8)	6.7 ± 0.9

[a] Values represent means plus or minus standard errors. [b] Equipotent molar ratio relative to carbamylcholine, which equals 1.00. [c] Dose required to cause half-maximal pupillary dilatation in mice. [d] Dose required to double the dose of oxotremorine inducing a predetermined (grade 2) tremor intensity in mice.

Table II. Dissociation Constants and Relative Efficacies of Some Oxotremorine Analogues at Muscarinic Receptors in the Guinea Pig Ileum

compd	n	K_A, μmol/L	rel efficacy[a]	% occupancy for 50% resp[b]
1	6	0.68 ± 0.19	1.00	3.5
(R)-14	5	2.34 ± 0.42	0.29 ± 0.06	11.0
16[c]	5	22.9 ± 2.7	6.51 ± 0.71	0.55

[a] Calculated from the K_A and ED_{50} values as described in ref 4. [b] Calculated as $100ED_{50}/(K_A + ED_{50})$. [c] From ref 23.

pig ileum. This stimulatory effect was inhibited by atropine (10^{-8} M) but was unaffected by hexamethonium (3×10^{-4} M), confirming the muscarinic nature of the response. The dose–response curves were similar in shape and maximum response to those of carbachol and oxotremorine, indicating full agonist activity. (R)-14 and (R)-15 were about 16-fold more active than the corresponding S enantiomers. Furthermore, (R)-14 was twice as active as (±)-14. N-Methylation of (R)-14 to give (R)-15 increased muscarinic activity almost 4-fold.

ED_{50} values of muscarinic agonists in the guinea pig ileum give no direct information on agonist affinities because of the existence of spare receptors. The dissociation constant (K_A) of (R)-14 at ileal muscarinic receptors, determined after elimination of spare receptors with propylbenzilylcholine mustard, was 3.4-fold greater than that similarly estimated for 1 (Table II). The efficacy of (R)-14 was less than one-third of that estimated for 1. Thus, the 11-fold lower parasympathomimetic activity of (R)-14 as compared to 1 was due to both lower affinity and lower efficacy of (R)-14. As a result of its lower efficacy, (R)-14 must occupy a greater fraction of the receptor pool to produce the same pharmacological response as 1 (Table II).

The enantiomers of 13 exhibited no agonist properties in the guinea pig ileum but showed some potentiation of carbachol responses at low concentrations. At higher concentrations, this action was replaced by competitive antagonism. Thus, the Schild plots[16] were linear at dose ratios greater than about 3, and the slopes of the regression lines were close to unity. (R)-13 was 16-fold more potent than (S)-13 and about twice as potent as (±)-13 (Table I).

Frog Rectus Abdominis. The enantiomers of 14 caused no contraction of the frog rectus abdominis muscle, whereas the enantiomers of 13 and 15 elicited slow contractions similar to those observed with carbachol. As previously noted[17] for numerous oxotremorine analogues, the quaternary compound 15 was considerably more active than the tertiary amine 13. No stereoselectivity was observed with respect to nicotinic effects on the frog rectus (Table I).

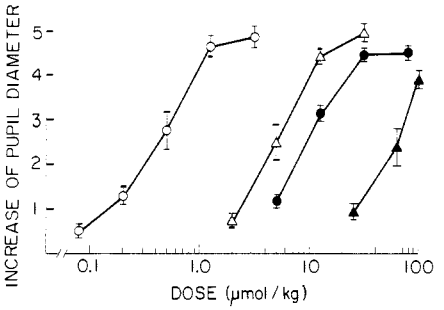


Figure 1. Mydriatic activity of atropine (○), (R)-13 (△), (±)-13 (●), and (S)-13 (▲) in mice. Mydriatic activity is expressed as increase of pupil diameter in arbitrary units relative to control mice whose pupil diameter was about 1 unit. Vertical bars represent standard errors of five measurements.

Antimuscarinic Activity in Intact Mice. None of the new compounds produced tremor in mice. The enantiomers of the tertiary amines 13 and 14 were tested for antagonism of oxotremorine-induced tremor to assess their central antimuscarinic potency. The R enantiomers of both compounds were about 20-fold more potent than the S enantiomers and twice as potent as the corresponding racemates (Table I).

The mydriatic activities in mice of the enantiomers and racemate of 13 as well as of atropine are shown in Figure 1. (R)-13 was twice as potent as (±)-13 (Table I). (S)-13 did not produce a maximal mydriatic response at doses below its LD_{50}.

Acute Toxicity in Mice. LD_{50} values of the new compounds and of oxotremorine are given in Table I. There was no correlation between the muscarinic or antimuscarinic activities of the enantiomers of 13–15 and their toxicities. For example, (S)-14 and (S)-15 were more than twice as toxic as their corresponding R enantiomers. Yet the latter were about 16-fold more active than the former as muscarinic stimulants in the guinea pig ileum. A significant ($r = 0.966$; $P = 0.007$) correlation was observed, however, between toxicity and nicotinic actions on the frog rectus for the five compounds that showed nicotinic effects. The enantiomers of 14, which had very low toxicity, were inactive as nicotinic stimulants.

(16) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol. Chemother.* **1959**, *14*, 48.

(17) Ringdahl, B. *Eur. J. Pharmacol.* **1984**, *99*, 177.

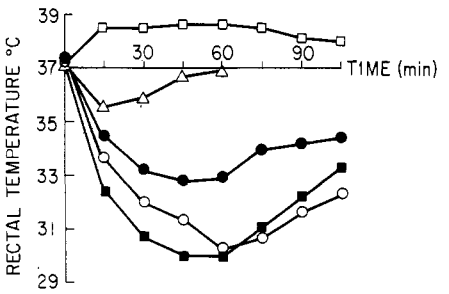


Figure 2. Time–response curves for hypothermia in mice induced by (*R*)-**14** (△, 2 μmol/kg; ●, 5 μmol/kg; ○, 8 μmol/kg) and by oxotremorine (■, 0.3 μmol/kg). □: saline-treated controls. Ambient temperature was 23 °C.

Discussion

The *R* enantiomers of **13–15** were more potent than the corresponding *S* enantiomers in their effects on the guinea pig ileum and as antimuscarinic agents in vivo. However, the observed enantiomeric potency ratios (14 to 21) in these tests are likely to be lower than the true ratios as the *S* enantiomers were not optically pure. In the LD_{50} determinations and in the effects on the frog rectus, the stereochemical impurity of the *S* enantiomers of **13–15** will not influence the results to any great extent since either no stereoselectivity was observed or the *S* enantiomers were the more potent.

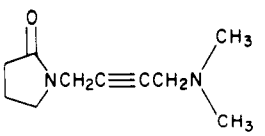
(*R*)-**13** was 5-fold more potent than atropine in antagonizing oxotremorine-induced tremor (a central effect) but had only $^1/_{11}$ of the mydriatic activity of atropine. Furthermore, (*R*)-**13** was 57.5-fold less potent than atropine on the guinea pig ileum. (*R*)-**13** therefore appears to be more selective in its central actions than atropine. Similar observations have been made[3,18–20] with other analogues of **1** and have been ascribed in part to their favorable distribution into the brain[21] because of low basicity and high lipophilicity and in part to their behavior as partial agonists with higher efficacy peripherally than centrally.[3] If potentiation of carbachol responses on the guinea pig ileum and weak salivation, observed with (*R*)-**13** in mice, are regarded as evidence of partial agonism, then the apparent central specificity of (*R*)-**13** can be similarly explained.

(*R*)-**14** seems to have greater efficacy than (*R*)-**13** peripherally since it was a full agonist in the guinea pig ileum, whereas (*R*)-**13** was an antagonist. Its efficacy at ileal muscarinic receptors, however, was only one-fourth to one-third that of **1** whose efficacy is low compared to those of many of its analogues[22,23] and compared to that of carbachol.[10] (*R*)-**14**, therefore, is expected to be a partial agonist or an antagonist at sites where there are few spare receptors. In addition to its agonist effects in the guinea pig ileum, (*R*)-**14** also showed clear peripheral parasympathomimetic activity in vivo as evidenced by pronounced salivation and lacrimation in mice. The efficacy of (*R*)-**14** centrally was not sufficient, however, to induce a tremor response and (*R*)-**14**, like (*R*)-**13**, antagonized oxotremorine-induced tremor. (*R*)-**14** nevertheless appears to have greater efficacy than (*R*)-**13** at other central sites since it, like **1**, but in contrast to (*R*)-**13** and (*S*)-**14**, caused profound hypothermia in mice (Figure 2), which is characteristic of central muscarinic stimulation.[24] This hypothermic effect was readily antagonized by atropine (5 μmol/kg, ip) but only marginally reduced by methylatropine (5 μmol/kg, ip). These observations suggest a larger receptor reserve with respect to hypothermic response in mice than with respect to tremorogenic response. (*R*)-**14** therefore appears to discriminate between different central muscarinic effects in vivo. The quaternary derivative (*R*)-**15** was a potent peripherally active parasympathomimetic but showed no apparent central effects. In comparison with the other compounds tested, (*R*)-**15** and (*S*)-**15** had relatively strong nicotinic actions and were quite toxic. The observation that the enantiomers of **13–15** did not differ in their potency on the frog rectus is in agreement with our previous suggestion that the 2-pyrrolidone moiety of oxotremorine analogues is relatively unimportant for nicotinic action.[17]

We have previously summarized evidence showing that dissociation constants at muscarinic receptors of agonist analogues of **1**, measured in the guinea pig ileum after elimination of spare receptors, and those of structurally related antagonists, measured in the ileum from inhibition of agonist-induced contractions, reflect affinities for a common receptor site and that they are directly comparable.[25] A comparison of the dissociation constant of the antagonist (*R*)-**13** (Table I) with that of the agonist **1** (Table II) shows that the methyl group of (*R*)-**13** increased the affinity for ileal muscarinic receptors 13.8-fold compared to **1**, i.e. by about the same amount (14.8-fold)[10] as the methyl group of (*R*)-**2** increased the affinity compared to **1**. A similar (14.5-fold) affinity difference was noted between (*R*)-**3** and its achiral, unsubstituted analogue.[10] It therefore appears that the methyl group of (*R*)-**13** participates in an additional interaction with the receptor, as has been previously suggested[4,10] for the methyl groups of (*R*)-**2** and (*R*)-**3**. Furthermore, the almost identical affinities of (*R*)-**13** and (*R*)-**2** (Table I) indicate interaction (presumably hydrophobic) with a common receptor locus.

The stereochemical requirements for muscarinic and antimuscarinic activity in vitro and in vivo among **13–15** were similar since the *R* enantiomers were consistently the more potent. This observation and the fact that **14** had similar enantiomeric potency ratio both as an agonist (guinea pig ileum) and as an antagonist (tremorolytic activity) suggest that binding to the receptor (affinity) is the prime determinant of stereoselectivity. A similar observation has been made previously[10,22] with other analogues of **1** that showed little or no stereoselectivity with respect to their receptor activating abilities (efficacy).

The methyl group of (*R*)-**14** decreased the efficacy 22-fold as compared to its unsubstituted analogue (**16**) (Table II). This methyl group also increased the affinity of **16**



16

9.8-fold, an increase that agrees well with the affinity difference between (*R*)-**13** and **1**. These results suggest that the methyl group of (*R*)-**14** also is involved in hydrophobic

(18) Dahlbom, R.; Karlēn, B.; George, R.; Jenden, D. J. *J. Med. Chem.* **1966**, *9*, 843.
(19) Dahlbom, R.; Karlēn, B.; George, R.; Jenden, D. J. *Life Sci.* **1966**, *5*, 1625.
(20) Karlēn, B.; Lindeke, B.; Lindgren, S.; Svensson, K.-G.; Dahlbom, R.; Jenden, D. J.; Giering, J. E. *J. Med. Chem.* **1970**, *13*, 651.
(21) Karlēn, B.; Jenden, D. J. *Res. Commun. Chem. Pathol. Pharmacol.* **1970**, *1*, 471.
(22) Ringdahl, B. *Br. J. Pharmacol.* **1984**, *82*, 269.
(23) Ringdahl, B. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 67.
(24) Lomax, P.; Jenden, D. J. *Int. J. Neuropharmacol.* **1966**, *5*, 353.
(25) Ringdahl, B. In "Dynamics of Cholinergic Function"; Hanin, I., Ed.; Plenum Press: New York, in press.

interaction. The agonist (*R*)-**14** and the antagonist (*R*)-**13** therefore seem to bind in an essentially identical manner to the receptor. In contrast, classical muscarinic antagonists are believed to interact predominantly with accessory sites adjacent to the agonist binding site of the receptor and display little similarity in their structural and stereochemical requirements with those of muscarinic agonists.[26]

Experimental Section

Melting points were determined in a heated metal block using open-glass capillaries and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and agreed with theoretical values within ±0.4%. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. ^{1}H NMR spectra were routinely recorded on a Varian T-60 instrument. In all cases, spectral data were consistent with the assigned structure. Merck silica gel 60 F_{254} analytical thin-layer chromatography (TLC) plates were used throughout this work. Except for dioxane, which was filtered over Al_2O_3 to remove peroxides, commercially available solvents were used without further purification.

(*S*)-5-Carbethoxy-2-pyrrolidone [(*S*)-8] was prepared from **6** as described previously.[13] The yield was 65% of a colorless oil [bp 148 °C (1 mmHg)] that crystallized on standing: mp 48–50 °C, $[\alpha]^{22}_D$ +6.3° (*c* 1, EtOH); lit.[13] mp 48–50 °C, $[\alpha]^{20}_D$ +2.4° (*c* 10, EtOH).

(*R*)-5-Carbethoxy-2-pyrrolidone [(*R*)-8]. Freshly distilled $SOCl_2$ (9.67 g, 81 mmol) was added during 10 min and at 0 °C to a suspension of 7 g (54 mmol) of **7** in 100 mL of absolute EtOH. The suspension was heated at 80 °C for 1.5 h and the solution concentrated in vacuo. The remaining yellow oil was purified by distillation: bp 138 °C (3 mmHg); yield 8 g (94%); $[\alpha]^{22}_D$ −6.0° (*c* 1, EtOH). The ^{1}H NMR spectra of (*R*)-**8** and (*S*)-**8** were identical and agreed with that previously published[13] for (*S*)-**8**.

(*S*)-5-(Hydroxymethyl)-2-pyrrolidone [(*S*)-9]. A solution of 7.7 g (49 mmol) of (*S*)-**8** in 10 mL of THF was added dropwise to a suspension of $LiBH_4$ (2.2 g, 100 mmol) in 75 mL of THF at 0 °C and under N_2. The viscous mixture was stirred at room temperature for 16 h. Then, 60 mL of 20% acetic acid was added to the cooled suspension. The THF was evaporated, and the remaining water solution was applied to an ion-exchange column (Dowex 50W-X, 200–400 mesh) to remove Li^+. The column was washed very carefully with 250 mL of water, which then was evaporated. Boric acid was removed from the resulting semisolid by evaporation with methanol (3 × 75 mL). The yellow oil thus obtained was distilled to give 4.4 g (78%) of a colorless oil that crystallized after several hours: bp 148 °C (0.04 mmHg), mp 65–68 °C, $[\alpha]^{22}_D$ +29° (*c* 1, EtOH); lit.[13] mp 66–68 °C, $[\alpha]^{20}_D$ +29° (*c* 5, EtOH).

(*R*)-5-(Hydroxymethyl)-2-pyrrolidone [(*R*)-9] was prepared similarly from (*R*)-**8** in 85% yield: mp 61–63 °C and $[\alpha]^{22}_D$ −26° (*c* 1, EtOH).

(*S*)-5-(Bromomethyl)-2-pyrrolidone [(*S*)-10] was synthesized from (*S*)-**9** as described previously.[13] However, a simplified procedure was used for purification of the oily product, primarily from contaminating $Ph_3P{=}O$. Thus, chromatography on a silica gel (28–200 mesh) column using CH_2Cl_2–MeOH (19:1) as eluent gave (*S*)-**10** as a white crystalline solid in 70% overall yield: mp 69–72 °C, $[\alpha]^{22}_D$ −32° (*c* 1, EtOH); lit.[13] mp 71–74 °C, $[\alpha]^{20}_D$ −33° (*c* 5, EtOH).

(*R*)-5-(Bromomethyl)-2-pyrrolidone [(*R*)-10] was prepared similarly from (*R*)-**9** in 75% yield: mp 67–69 °C; $[\alpha]^{22}_D$ +29° (*c* 1, EtOH).

(*R*)-5-Methyl-2-pyrrolidone [(*R*)-11]. A solution of (*S*)-**10** (5.6 g, 31.4 mmol), 10.9 g (37.5 mmol) of tributyltin hydride (Bu_3SnH), and 50 mg of azoisobutyronitrile (AIBN) in toluene (50 mL) was stirred for 4 h at 80 °C under N_2. The yellow liquid remaining after evaporation of the solvent was chromatographed on a column of silica gel (100 g). The Bu_3SnBr and the excess of Bu_3SnH were eluted with CH_2Cl_2. (*R*)-**11** was then eluted with CH_2Cl_2–MeOH (9:1). Evaporation of the solvents and distillation gave 2.3 g (74%) of product: bp 66 °C (3 mmHg), $[\alpha]^{22}_D$ +16.6° (*c* 1, EtOH); lit.[27] $[\alpha]^{20}_D$ +26.6° (*c* 0.9, H_2O).

(*S*)-5-Methyl-2-pyrrolidone [(*S*)-11] was prepared similarly from (*R*)-**10** in 61% yield: bp 66 °C (3 mmHg); $[\alpha]^{22}_D$ −14.1° (*c* 1, EtOH).

(*R*)-5-Methyl-*N*-2-propynyl-2-pyrrolidone [(*R*)-12]. A solution of (*R*)-**11** (2 g, 20.2 mmol) in benzene (10 mL) was slowly added to NaH (about 21 mmol, obtained by washing 0.85 g of a 60% suspension of NaH in mineral oil three times with hexane) in 25 mL of benzene. The mixture was heated at 80 °C for 1 h. Then, 3.1 g (21 mmol) of propargyl bromide (80% in toluene) was added over 20 min and at 15 °C with continuous stirring. All manipulations were made under N_2. The mixture was stirred at room temperature for 48 h and then filtered. The solid was washed with 100 mL of benzene, and the combined organic phases were concentrated in vacuo to give 2.3 g (83%) of a yellow oil that contained besides (*R*)-**12** also 10–15% of 5-methyl-*N*-allenyl-2-pyrrolidone. The oil was not further purified as the allene isomer did not interfere in the succeeding Mannich reaction.

(*S*)-5-Methyl-*N*-2-propynyl-2-pyrrolidone [(*S*)-12] was prepared similarly from (*S*)-**11** in 80% yield.

(*R*)-*N*-(4-Pyrrolidino-2-butynyl)-5-methyl-2-pyrrolidone [(*R*)-13], Oxalate Salt. A mixture of (*R*)-**12** (1.1 g, ~8 mmol), paraformaldehyde (0.3 g, 10 mmol), pyrrolidine (0.71 g, 10 mmol), and CuCl (30 mg) in 10 mL of dioxane was heated at 80 °C for 2 h. The dioxane was evaporated, and the oily residue was taken up in 30 mL of 1 N HCl. The water solution was extracted with CH_2Cl_2 (3 × 30 mL). This extraction effectively removed the above-mentioned allenic isomer. K_2CO_3 was then added to adjust the pH to 10. Extraction with CH_2Cl_2 (3 × 30 mL), drying (K_2CO_3), and evaporation yielded a brown oil that was purified by chromatography on a silica gel column with CH_2Cl_2–CH_3OH (9:1) as eluent. The yield was 0.8 g (49%) of a yellow oil. This oil was converted to the oxalate, which was recrystallized from ethanol–ether: mp 116–118 °C; $[\alpha]^{22}_D$ +37.8° (*c* 1, EtOH). Anal. ($C_{15}H_{22}N_2O_5$) C, H, N.

(*S*)-*N*-(4-Pyrrolidino-2-butynyl)-5-methyl-2-pyrrolidone [(*S*)-13], oxalate salt was prepared similarly from (*S*)-**12**: yield 45%; mp 107–111 °C; $[\alpha]^{22}_D$ −32.9° (*c* 1, EtOH). Anal. ($C_{15}H_{22}N_2O_5$) C, H, N.

(*R*)-*N*-[4-(Dimethylamino)-2-butynyl]-5-methyl-2-pyrrolidone [(*R*)-14], Sesquioxalate Salt. A mixture of dimethylamine (3 mL), which was condensed at −5 °C into a 20-mL round-bottom flask, (*R*)-**12** (1.1 g, ~8 mmol), paraformaldehyde (0.3 g, 10 mmol), and CuCl (30 mg) in 10 mL of dioxane was stirred in the sealed flask under N_2 for 48 h at room temperature. The product was then isolated as described above for (*R*)-**13**, yielding 0.65 g (45%) of a yellow oil. This oil was converted to the sesquioxalate, which was recrystallized from ethanol–ether: mp 119–121 °C; $[\alpha]^{22}_D$ +37.5° (*c* 1, EtOH). Anal. ($C_{14}H_{21}N_2O_7$) C, H, N.

(*S*)-*N*-[4-(Dimethylamino)-2-butynyl]-5-methyl-2-pyrrolidone [(*S*)-14], sesquioxalate salt was prepared similarly from (*S*)-**12**: yield 40%; mp 118–120 °C; $[\alpha]^{22}_D$ −31.9° (*c* 1, EtOH). Anal. ($C_{14}H_{21}N_2O_7$) C, H, N.

(*R*)-*N*-[4-(Dimethylamino)-2-butynyl]-5-methyl-2-pyrrolidone [(*R*)-15], methiodide salt, was prepared by adding an excess of MeI to a solution of the free base of (*R*)-**14** (0.2 g, 1 mmol) in Et_2O. The oily precipitate was crystallized from ethanol–ether: yield 0.25 g (74%); mp 131–133 °C; $[\alpha]^{22}_D$ +20.1° (*c* 1, EtOH). Anal. ($C_{12}H_{21}IN_2O$) C, H, N.

(*S*)-*N*-[4-(Dimethylamino)-2-butynyl]-5-methyl-2-pyrrolidone [(*S*)-15], methiodide salt was prepared similarly from the free base of (*S*)-**14**: yield 80%; mp 127–130 °C; $[\alpha]^{22}_D$ −18.1° (*c* 1, EtOH). Anal. ($C_{12}H_{21}IN_2O$) C, H, N.

Isolated Guinea Pig Ileum. Male guinea pigs (Hartley, 350–400 g) were killed by a blow to the head and bled. Segments of the ileum were removed and suspended in a 10-mL organ bath containing Tyrode solution (pH 7.4) at 37 °C and gassed with O_2 containing 5% CO_2. Hexamethonium (3 × 10^{-4} M) was included in the Tyrode solution. Contractions were recorded isotonically at 1 g of tension on an electromechanical displacement transducer and a potentiometric recorder. Agonists were compared on the same preparation with carbachol by using the cumulative dose–

(26) Ariens, E. J.; Simonis, A. M. *Ann. N.Y. Acad. Sci. U.S.A.* **1967**, *144*, 842.
(27) Cervinka, O.; Fabriova, A.; Novak, V. *Collect. Czech. Chem. Commun.* **1965**, *30*, 1742.

response technique. Agonist potencies were expressed as ED_{50} values, obtained by interpolation at the 50% response level, or as equipotent molar ratios (EPMR) relative to carbachol. The preparation was allowed to equilibrate with each concentration of antagonist for 20 min before dose–response curves to carbachol were obtained. Affinity constants (K_B) of antagonists were calculated from

$$K_B = [\text{antagonist}]/\text{dose ratio} - 1$$

where the dose ratio is the ratio of equieffective concentrations of carbachol after and before the addition of antagonist. The antagonism was shown to be competitive by the method of Arunlakshana and Schild.[16]

Dissociation constants (K_A) and relative efficacies of oxotremorine and (*R*)-14 at muscarinic receptors of isolated guinea pig ileum were determined by the method of Furchgott and Bursztyn[28] as modified by Ringdahl.[10] Control dose–response curves to 1 and (*R*)-14 were constructed on the same preparation. The ileum was then treated with propylbenzilylcholine mustard (PrBCM) at 2×10^{-8} M for 15–20 min. This treatment reduced the maximal responses to 1 and (*R*)-14 by 20–80%. Double-reciprocal plots of equieffective concentrations of each agonist before and after treatment with PrBCM gave straight lines whose slopes and intercepts were used to calculate K_A values of the agonist–receptor complexes.[28] The efficacy of (*R*)-14 relative to that of 1 was calculated from the respective K_A and ED_{50} values.[4,10]

Frog Rectus Abdominis. A frog rectus abdominis preparation from *Rana pipiens* was set up at 20 °C in a 10-mL organ bath containing aerated Clark–Ringer solution (pH 7.4) as described previously.[29] Contractions were recorded as described above for the guinea pig ileum. The preparation was exposed to each drug concentration for 5 min. Equipotent molar ratios relative to carbachol were determined in three-point assays.[29]

Mydriatic Activity in Mice. Mydriatic activity was estimated by measuring the pupillary diameter of Swiss–Webster male mice weighing 25–32 g in groups of five, both before and 15 min after the intraperitoneal injection of the test compound. The measurements were made under standard lighting conditions with a binocular dissecting microscope fitted with a calibrated eyepiece. ED_{50} values were obtained from the dose–response curves by interpolation at the 50% response level.

Tremorolytic Activity in Mice. The median dose of oxotremorine required to evoke an intermittent spontaneous (grade 2) tremor[30] was estimated by the up and down method for small samples described by Dixon.[31] Oxotremorine was administered intravenously in a logarithmic series of doses with a spacing of 0.1 unit in the log scale. Four linearly spaced doses including zero of each antagonist tested were given intraperitoneally to groups of six or more mice 15 min before oxotremorine administration. The presence or absence of tremors was determined by visual inspection during the first 5 min after administration of oxotremorine. The median effective dose of oxotremorine was plotted against the dose of the antagonist. That dose of antagonist that doubled the median effective dose of oxotremorine was estimated by linear regression analysis.[5,20]

Hypothermic Effect in Mice. The test compounds were administered intravenously to groups of five mice at five dose levels. One group was treated with 0.9% NaCl. Rectal temperature was recorded with a digital thermometer (Yellow Springs Instruments, Model 49TA) at constant ambient temperature (23 ± 0.5 °C).

Acute Toxicity in Mice. LD_{50} values were determined by intravenous injection using the up and down method for small samples.[31] Mortality counts were taken at 15 min.

Acknowledgment. This research was supported by USPHS Grant MH-17691. We thank Nelly Canaan for skillful secretarial assistance.

Registry No. **1**, 70-22-4; (**R**)-**2**, 54139-69-4; (*S*)-**2**, 54139-73-0; **6**, 56-86-0; **7**, 4042-36-8; (*S*)-**8**, 7149-65-7; (*R*)-**8**, 68766-96-1; (*S*)-**9**, 17342-08-4; (*R*)-**9**, 66673-40-3; (*S*)-**10**, 72479-05-1; (*R*)-**10**, 98612-60-3; (*R*)-**11**, 21395-93-7; (*S*)-**11**, 1558-60-7; (*R*)-**12**, 98612-61-4; (*S*)-**12**, 98634-06-1; (*R*)-**13**, 98673-88-2; (*S*)-**13**, 98673-89-3; (+−)-**13**, 72314-33-1; (*R*)-**13** oxalate, 98673-91-7; (*S*)-13 oxalate, 98674-78-3; (*R*)-**14**, 98612-62-5; (*S*)-**14**, 98612-63-6; (+−)-**14**, 98673-90-6; (*R*)-**14** $^3/_2$ oxalate, 98612-66-9; (*S*)-**14** $^3/_2$ oxalate, 98612-67-0; (*R*)-**15**, 98612-64-7; (*S*)-**15**, 98612-65-8; (*R*)-5-methyl-*N*-allenyl-2-pyrrolidone, 98612-68-1; (*S*)-5-methyl-*N*-allenyl-2-pyrrolidone, 98612-69-2.

(28) Furchgott, R. F.; Bursztyn, P. *Ann. N.Y. Acad. Sci. U.S.A.* **1967**, *144*, 882.
(29) Edinburgh Pharmacology Department Staff. "Pharmacological Experiments on Isolated Preparations"; Livingstone: Edinburgh, 1968; pp 13, 38.
(30) Cho, A. K.; Jenden, D. J. *Int. J. Neuropharmacol.* **1964**, *3*, 27.
(31) Dixon, W. J. *J. Am. Stat. Assoc.* **1965**, *60*, 967.