animal to an absorbent paper tissue at intervals during the first 10 min after drug administration. The presence or absence of intermittent spontaneous (grade 2) tremor[22] was determined by visual inspection.

Analgesic activity was estimated in the tail-flick assay[23] by the up-and-down method.[21] After determination of control reaction times, drugs were administered ip, and posttreatment reaction times were recorded 10 min later. Animals that had posttreatment reaction times at least two times greater than the control reaction time were regarded as responders.

Acute Toxicity. LD_{50} values were determined by iv injection and the up-and-down method.[21] Mortality counts were taken at 1 h.

Acknowledgment. This work was supported by U.S. Public Health Service Grants GM37816 and MH17691.

Registry No. **3**, 110797-76-7; **4**, 110797-77-8; **5**, 110797-78-9; **6**, 110825-65-5; **7**, 110797-79-0; **8**, 110797-80-3; **9**, 110797-81-4; **10**, 110797-82-5; $HO(CH_2)_3NHMe$, 42055-15-2; $HO(CH_2)_4NHMe$, 42042-68-2; *N*-(2-propynyl)-2-pyrrolidone, 766-61-0; *N*-(2-propynyl)-5-methyl-2-pyrrolidone, 18327-34-9.

(22) Cho, A. K.; Jenden, D. J. *Int. J. Neuropharmacol.* **1964**, *3*, 27.
(23) D'Amour, F. E.; Smith, D. L. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74.

Dimethylsulfonium and Thiolanium Analogues of the Muscarinic Agent Oxotremorine

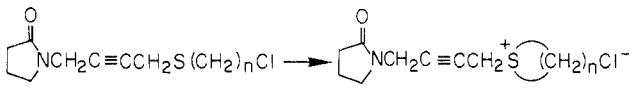
Björn Ringdahl

Department of Pharmacology, School of Medicine, University of California, Los Angeles, California 90024-1735.
Received June 26, 1987

Dimethylsulfonium (**6a** and **6b**) and thiolanium analogues (**7a** and **7b**) of oxotremorine were synthesized and found to be potent muscarinic agents in vivo and in vitro. Compound **6a** exceeded oxotremorine in potency. Their affinities for muscarinic receptors in the guinea pig ileum and urinary bladder, estimated pharmacologically, were higher than those of the corresponding trimethylammonium (**8a** and **8b**) and *N*-methylpyrrolidinium compounds (**9a** and **9b**). However, the new compounds had lower intrinsic efficacies than their quaternary ammonium analogues. The compounds also had high affinity for central muscarinic receptors as measured by displacement of specifically bound (−)-[^{3}H]-*N*-methylscopolamine from homogenates of the rat cerebral cortex. Half-maximal occupation of cortical muscarinic receptors by **6a**, **6b**, **7a**, and **7b** was achieved at concentrations of 0.8, 5.4, 0.3, and 3.3 μM, respectively. The competition curves of **6a**, **6b**, and **7a** were adequately described by a two-site binding equation. The ratio of low- and high-affinity dissociation constants agreed with relative efficacy estimated on the ileum. The thiolanium salt **7a** was a fairly potent nicotinic agent on the frog rectus abdominis.

Recently, we have synthesized a series of 2-haloalkylamines related to the muscarinic agent oxotremorine, *N*-(4-pyrrolidino-2-butynyl)-2-pyrrolidone.[1,2] When administered in vivo, these compounds penetrate readily into the central nervous system and produce profound muscarinic effects, e.g., tremor and analgesia.[3,4] These effects and the persistent antimuscarinic actions that follow the initial stimulation may be ascribed to the aziridinium ions formed by in vivo cyclization of the parent 2-haloalkylamines.

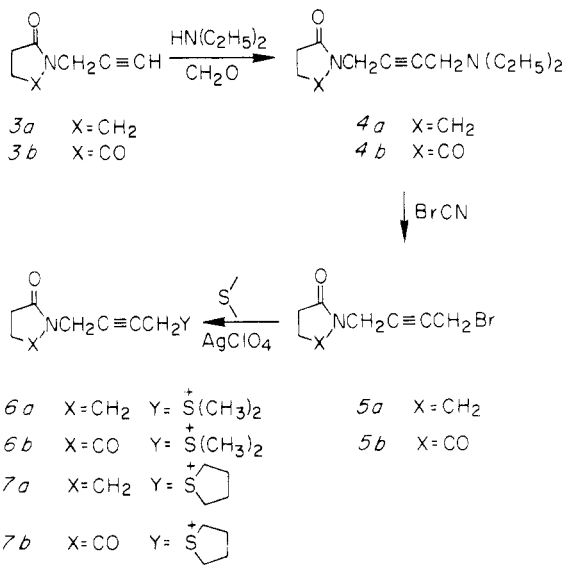
Chloroalkyl sulfides are known to cyclize to sulfonium ions.[5] It seemed possible that chloroalkyl sulfide analogues **1** of oxotremorine, like the corresponding 2-haloalkylamines, would be useful carriers for the passage of charged muscarinic compounds into the central nervous system. Before extensive studies of such chloroalkyl



1 **2**

sulfides were initiated, it was desirable to characterize the pharmacological properties of sulfonium analogues of oxotremorine. This report describes the muscarinic and nicotinic actions of dimethylsulfonium (**6a** and **6b**) and thiolanium analogues (**7a** and **7b**) of oxotremorine (Scheme I). Compound **6a** closely resembles the episulfonium ion **2** (n = 2) derived from the 2-chloroethyl sulfide **1** (n = 2), whereas **7a** is the cyclization product of the 4-chlorobutyl sulfide **1** (n = 4).

Scheme I



Chemistry. The synthesis of compounds **6a**, **6b**, **7a**, and **7b** is outlined in Scheme I. *N*-2-Propynyl-2-pyrrolidone (**3a**) and *N*-2-propynylsuccinimide (**3b**) were converted to the Mannich bases **4a** and **4b**, respectively. Treatment of

(1) Ringdahl, B.; Resul, B.; Ehlert, F. J.; Jenden, D. J.; Dahlbom, R. *Mol. Pharmacol.* **1984**, *26*, 170.
(2) Ringdahl, B.; Jenden, D. J. *J. Med. Chem.* **1987**, *30*, 852.
(3) Ringdahl, B.; Jenden, D. J. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 370.
(4) Russell, R. W.; Crocker, A. D.; Booth, R. A.; Jenden, D. J. *Psychopharmacology (Berlin)* **1986**, *88*, 24.
(5) Lowe, P. A. *The Chemistry of the Sulphonium Group*; Stirling, C. J. M., Patai, S., Eds.; Wiley: Chichester, 1981; Chapter 11, p 267.

0022-2623/88/1831-0164$01.50/0

Table I. Muscarinic Activity on the Guinea Pig Ileum and Urinary Bladder and Sialagogic Activity in Mice of Sulfonium Analogues of Oxotremorine[a]

compound	muscarinic act. in vitro: tissue	n	EC_{50}, μM	EPMR[b]	K_D,[c] μM	rel. efficacy	sialagogic act.:[d] ED_{50}, μmol/kg
6a	ileum	9	0.029 ± 0.004	0.24 ± 0.01	1.5 ± 0.3	0.41 ± 0.06	0.024 ± 0.003
	bladder	4	0.20 ± 0.05	0.32 ± 0.02	1.3 ± 0.4	0.48 ± 0.08	
6b	ileum	6	0.41 ± 0.05	4.1 ± 0.4	6.9 ± 0.8	0.11 ± 0.02	0.48 ± 0.07
	bladder	4	1.7 ± 0.3	3.7 ± 0.1	5.9 ± 2.1	0.15 ± 0.02	
7a	ileum	8	0.25 ± 0.02	2.1 ± 0.1	1.3 ± 0.1	0.054 ± 0.005	0.35 ± 0.05
	bladder[e]	4	1.2 ± 0.2	2.3 ± 0.4	1.1 ± 0.1	0.057 ± 0.005	
7b	ileum[f]	6	7.9 ± 1.4	81 ± 12	7.5 ± 2.3	0.0061 ± 0.001	*g*
	bladder[h]	4			13.1 ± 1.2		
8a	ileum[i]	5	0.023 ± 0.004		2.9 ± 0.1	0.95 ± 0.04	0.058 ± 0.008[j]
	bladder[i]	6	0.37 ± 0.03		4.3 ± 0.7	0.88 ± 0.12	
carbachol	ileum	6	0.11 ± 0.01	1.0	15.4 ± 3.1[i]	1.0	0.083 ± 0.012
	bladder	8	0.62 ± 0.10	1.0	17.0 ± 2.1[i]	1.0	

[a] All values are means ± standard errors; n equals the number of tissues used. [b] Equipotent molar ratio. [c] Dissociation constant of drug–receptor complex. [d] Compounds administered intravenously. [e] Maximum response was 54 ± 8% of that elicited by carbachol. [f] Maximum response was 45 ± 7% of that elicited by carbachol. [g] No salivation observed at doses below the LD_{50} value. [h] Antagonist to carbachol. [i] Value from ref 8. [j] Value from ref 3.

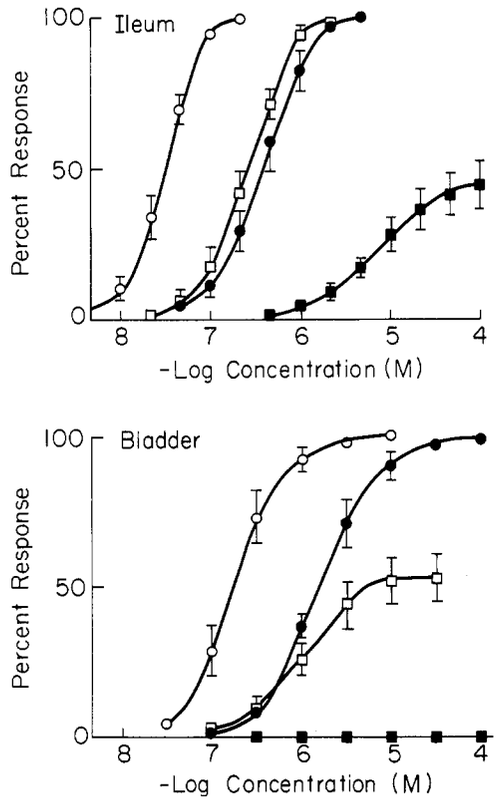


Figure 1. Concentration–response curves of **6a** (○), **6b** (●), **7a** (□), and **7b** (■) in strips of the guinea pig ileum and urinary bladder. Responses are expressed relative to the maximum response elicited by carbachol. Vertical bars show standard errors. Number of tissues used is given in Table I.

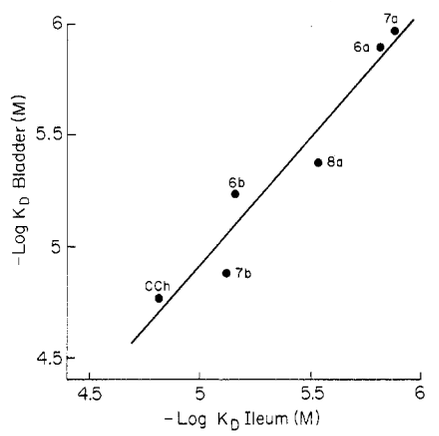


Figure 2. Relationship between negative logarithms of the dissociation constants (pK_D) of **6a**, **6b**, **7a**, **7b**, **8a**, and carbachol (CCh) at muscarinic receptors in the guinea pig ileum and urinary bladder. The regression line is described by pK_D (bladder) = –0.69 ± (1.12 ± 0.15)pK_D (ileum) (t_4 = 7.6; $P < 0.005$).

4a and **4b** with cyanogen bromide in a von Braun reaction yielded the propargylic bromides **5a** and **5b**. Alkylation of dimethyl sulfide or tetrahydrothiophene by **5a** and **5b** in the presence of $AgClO_4$ gave the desired sulfonium compounds as perchlorate salts in 53–73% yield. Attempts to prepare the bromide salts by alkylation in the absence of $AgClO_4$ were unsuccessful.

Pharmacological Results

Muscarinic Activity. Compounds **6a**, **6b**, and **7a** elicited full contractile responses compared to carbachol on the isolated guinea pig ileum, whereas **7b** produced a submaximal response (Figure 1). The dimethylsulfonium analogue **6a** was somewhat more potent than oxotremorine and 4-fold more potent than carbachol (Table I). It thus belongs to the most potent muscarinic agonists known.

The effects of **6a** were antagonized in a competitive manner by *N*-methylatropine. In three experiments, a concentration of 0.05 μM of *N*-methylatropine caused a (96 ± 14)-fold dextral shift of the concentration–response curve to **6a**. Hexamethonium (300 μM) had no apparent effect on the potency of **6a**. These results show that the contractile responses of **6a** on the ileum were due to muscarinic effects. The high potency of **6a** and its congeners may be ascribed primarily to high affinity for muscarinic receptors (low K_D values). Their intrinsic efficacies were substantially lower than that of carbachol (Table I).

The dimethylsulfonium analogues **6a** and **6b** elicited a full contractile response on the guinea pig urinary bladder, whereas the thiolanium salts were partial agonists (**7a**) and antagonists (**7b**). The EC_{50} values of **6a**, **6b**, and **7a** as well as those of carbachol and **8a** were significantly ($P < 0.001$) greater on the bladder than on the ileum (Table I). In spite of the observed differences in agonist potency and relative maximal responses between the ileum and bladder (Figure 1), the equipotent molar ratio, dissociation constant, and relative efficacy of each compound showed good agreement in the two tissues. Plots of K_D values and relative efficacies estimated on the ileum versus those obtained on the bladder gave linear regressions with slopes that were not significantly different from 1 (Figure 2). Furthermore, the data points tended to fall on the line of equivalence.

All of the compounds except **7b** induced salivation in mice (Table I). Sialagogic potency (ED_{50}) was correlated

Table II. Nicotinic Activity in Vitro and Acute Toxicity in Mice of Sulfonium Analogues of Oxotremorine[a]

compound	frog rectus abdominis		selectivity: EPMR (rectus)/EPMR (ileum)	acute toxicity:[c]
	EC_{50}, μM	EPMR[b]		LD_{50}, μmol/kg
6a	29.0 ± 4.9 (4)	8.1 ± 1.3 (6)	33.8	2.8 ± 0.4
6b	2600 ± 320 (4)[d]	692 ± 67 (6)	169	70 ± 10
7a	5.0 ± 1.8 (5)	1.4 ± 0.3 (5)	0.66	2.2 ± 0.3
7b	66.2 ± 7.5 (5)	18 ± 1.8 (7)	0.22	3.5 ± 0.5
8a		2.1 ± 0.1 (8)[e]	7.0[e]	1.0 ± 0.1[f]
carbachol	3.8 ± 0.6 (10)	1.00	1.00	1.7 ± 0.2

[a] Values are means ± standard errors. Number of test preparations used is given in parentheses. [b] Equipotent molar ratio. [c] Compounds given intravenously. [d] Maximum response was lower than that of carbachol. [e] Value from ref 16. [f] Value from ref 3.

Table III. Muscarinic Receptor Binding of Sulfonium Analogues of Oxotremorine in Rat Cerebral Cortex[a]

no.	IC_{50} (corrected), μM	high-affinity sites, %	K_H, μM	K_L, μM	K_L/K_H
6a	0.80 ± 0.01	28.4 ± 2.2	0.011 ± 0.003	2.2 ± 0.3	196 ± 41
6b	5.4 ± 0.3	27.4 ± 1.9	0.19 ± 0.07	12.7 ± 1.5	67 ± 24
7a	0.31 ± 0.02	37.0 ± 3.0	0.069 ± 0.01	0.99 ± 0.07	14 ± 3
7b	3.3 ± 0.2		4.1 ± 0.4		
8a	1.2 ± 0.05	31.3 ± 2.2	0.019 ± 0.005	5.4 ± 0.4	286 ± 72

[a] Values are means ± standard errors from three independent estimates, each done in triplicate.

($r = 0.94$; $P < 0.025$) with spasmogenic activity (EC_{50}) on the ileum. The calculations were made on negative logarithms of molar doses and concentrations.

Nicotinic Activity. Compounds **6a**, **7a**, and **7b** elicited maximal responses similar to that of carbachol on the frog rectus abdominis muscle. In contrast, **6b** was a weak partial agonist. As observed on the ileum, the 2-pyrrolidones **6a** and **7a** were more potent than the corresponding succinimides **6b** and **7b**. However, in contrast to the results on the ileum, the dimethylsulfonium salts **6a** and **6b** were less potent than the corresponding thiolanium salts **7a** and **7b**. Selectivity for muscarinic as opposed to nicotinic receptors was expressed as the ratio of equipotent molar ratios obtained on the frog rectus and the guinea pig ileum (Table II). Compared to carbachol, the dimethylsulfonium salts were highly selective for muscarinic receptors, whereas the thiolanium salts were somewhat less selective than carbachol. There was a significant correlation ($r = 0.95$; $P < 0.025$) between nicotinic activity on the frog rectus and acute toxicity in mice. No such correlation was observed between muscarinic activity and toxicity.

Muscarinic Receptor Binding. The sulfonium analogues displaced specifically bound (−)-[^{3}H]-*N*-methylscopolamine ([^{3}H]NMS) from rat cerebral cortex in an apparently competitive manner (Figure 3). The rank order of potency, as estimated from the IC_{50} values (corrected for receptor occupancy by [^{3}H]NMS) (Table III), was different from that observed for contractile activity on the guinea pig ileum. However, IC_{50} values obtained in the cortex were correlated ($r = 0.96$; $P < 0.025$) with K_D values estimated on the ileum. The competition curve of **7b** was adequately described by a one-site binding equation. For the other analogues, analysis of variance revealed a significant reduction in residual error when the data were fitted to a two-site model as compared with a one-site model. The high-affinity dissociation constants (K_H) generally agreed with the EC_{50} values for contraction of the ileum, whereas the low-affinity dissociation constants (K_L) were similar in magnitude to the K_D values estimated on the ileum and bladder. Furthermore, the ratio K_L/K_H was highly correlated ($r = 0.98$; $P < 0.005$) with agonist efficacy.

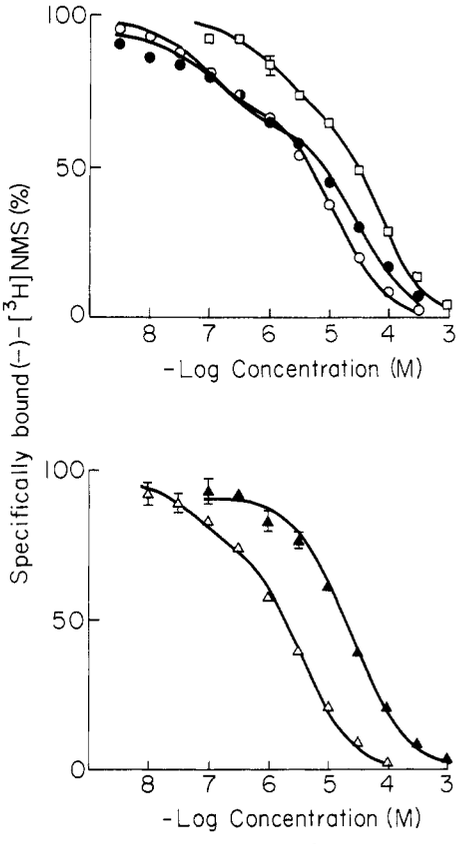
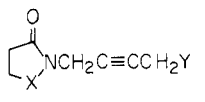


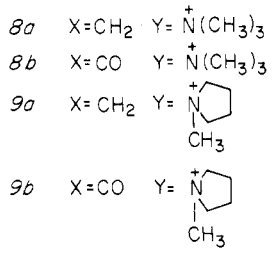
Figure 3. Competitive inhibition of (−)-[^{3}H]NMS binding in rat cerebral cortex by **8a** (●), **6a** (○), **6b** (□), **7a** (△), and **7b** (▲). Values are means ± standard errors from three experiments, each performed in triplicate. The concentration of (−)-[^{3}H]NMS used was 0.3 nM. The theoretical curves are the least-squares fit to the data.

Discussion

The in vitro muscarinic potencies of the dimethylsulfonium (**6a** and **6b**) and thiolanium salts (**7a** and **7b**) were very similar to those of the corresponding trimethylammonium (**8a** and **8b**) and *N*-methylpyrrolidinium salts (**9a** and **9b**).[6-8] However, the sulfonium compounds consistently had higher affinity and lower intrinsic efficacy at muscarinic receptors than the corresponding ammonium derivatives. In agreement with this observation, **7b** was a partial agonist and an antagonist, respectively, on the ileum and the bladder, whereas its ammonium analogue

(6) Ringdahl, B. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 67.
(7) Ringdahl, B. *Eur. J. Pharmacol.* **1987**, *140*, 13.
(8) Ringdahl, B. *Mol. Pharmacol.* **1987**, *31*, 351.





9b was a full agonist and partial agonist, respectively, in these tissues.[7] We have found previously that analogues of oxotremorine that have high affinity and low efficacy at muscarinic receptors may display selectivity based on tissue differences in muscarinic receptor reserve.[8,9] The thiolanium salt **7a** had about the same affinity as oxotremorine,[6-8] but 2.5-fold lower efficacy, and therefore appears to fulfill the above requirements for potentially selective muscarinic actions. Accordingly, it was a potent full agonist on the ileum, but only a partial agonist on the bladder (Figure 1).

Because of the linearity of the 2-butynyl chain of **6–9** and the resulting spatial separation of the cationic head from the 2-pyrrolidone and succinimide moieties, the sulfonium salts are not expected to differ much from the quaternary ammonium salts in their conformational properties. Furthermore, the dimethylsulfonium group is similar in size to the trimethylammonium group but has greater hydrophobicity.[10] Molecular orbital calculations have shown that the charge distribution of the cationic head of sulfonium compounds is quite different from that of the corresponding ammonium compounds. In sulfonium compounds, the sulfur atom appears to retain about one-fourth of the formal positive charge. The nitrogen atom of ammonium compounds, however, is essentially neutral, the positive charge being dispersed over adjacent carbon and hydrogen atoms.[11-13] Perhaps such differences in charge distribution are responsible for the higher affinity and lower intrinsic efficacy of **6a**, **6b**, **7a**, and **7b** as compared to the corresponding ammonium compounds.

Compound **6a** exceeded oxotremorine and acetylcholine in muscarinic potency. The high muscarinic activity of **6a** is somewhat surprising in view of the low potency of the dimethylsulfonium analogue of acetylcholine.[14] However, some thianium analogues of acetylcholine, e.g., *cis*-4-acetoxy-1-methylthianium iodide, were fairly potent muscarinic agonists.[11] The ability of **6a** and its congeners to displace effectively (−)-[^{3}H]NMS from muscarinic receptors confirms that they act directly at the receptor rather than through released acetylcholine. In general, the IC_{50} values (corrected) obtained in the cortex were somewhat lower than the K_D values estimated pharmacologically on the ileum and bladder. However, quantitative agreement between the two estimates of affinity is not expected because of the different conditions used in the receptor binding and pharmacological experiments. Like other muscarinic agonists,[15] **6a**, **6b**, and **7a** recognized high- and low-affinity states of cortical muscarinic receptors. This observation suggests that they possess efficacy at these receptors.

At nicotinic receptors of the frog rectus, the dimethylsulfonium analogues **6a** and **6b** were decidedly less potent than the trimethylammonium salts **8a** and **8b**.[16] In contrast, the thiolanium derivatives **7a** and **7b** were more potent than their *N*-methylpyrrolidinium analogues **9a** and **9b** (B. Ringdahl, unpublished results).

In conclusion, sulfonium analogues of oxotremorine are potent muscarinic agonists having high affinity for central and peripheral muscarinic receptors. Chloroalkyl sulfides such as **1**, therefore, appear to deserve evaluation as prodrugs of centrally active muscarinic agents. Compounds of this type may be useful as affinity labels for muscarinic receptors (cf. corresponding 2-haloethylamines)[1,17] or as muscarinic agonists having long-lasting effects selectively in the central nervous system. The latter would result from restricted diffusion of charged compounds such as **7a** across the blood–brain barrier into the periphery.[18]

Experimental Section

Melting points are uncorrected and were determined in a heated metal block using glass capillaries. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and, unless otherwise indicated, agreed with theoretical values within ±0.4%. Mass spectra were recorded on a Hewlett-Packard 5981A mass spectrometer at 70 eV. ^{1}H NMR spectra were obtained at 40 °C on a Bruker WP 200 spectrometer at 200 MHz. Chemical shifts are reported in parts per million (δ) downfield from internal $(CH_3)_4Si$ standard.

[4-(2-Oxopyrrolidinyl)-2-butynyl]dimethylsulfonium Perchlorate (6a). *N*-(4-Bromo-2-butynyl)-2-pyrrolidone (**5a**) was synthesized from *N*-[4-(diethylamino)-2-butynyl]-2-pyrrolidone (**4a**) as described previously.[19] A solution of 1.5 g (6.9 mmol) of **5a** in anhydrous CH_3CN (15 mL) was added dropwise at 0 °C to a solution of 0.47 g (0.56 mL, 7.6 mmol) of $(CH_3)_2S$ and 1.3 g (6.3 mmol) of anhydrous $AgClO_4$ in CH_3CN (50 mL). The reaction mixture was left at room temperature for 24 h. The AgBr was filtered off, and the CH_3CN was evaporated under vacuum. The residue was triturated twice with anhydrous ether and crystallized from acetone–ether to give 1.2 g (65%) of **6a** as white crystals: mp 113–114 °C; ^{1}H NMR (CD_3CN) δ 4.14 (s, 4 H, $CH_2C{\equiv}CCH_2$), 3.43 (t, J = 6.8 Hz, 2 H, 5-CH_2), 2.80 (s, 6 H, CH_3), 2.26 (t, $J \sim$ 7.6 Hz, 2 H, 3-CH_2), 1.90–2.10 (m, 2 H, 4-CH_2); MS, *m/e* (relative intensity) 137 (57), 136 (100), 98 (70). Anal. ($C_{10}H_{16}ClNO_5S$) C, H, N.

[4-(2-Oxopyrrolidinyl)-2-butynyl]thiolanium perchlorate (7a) was prepared similarly from **5a** (2.0 g, 9.3 mmol), tetrahydrothiophene (0.82 g, 9.3 mmol), and $AgClO_4$ (1.8 g, 8.7 mmol). The reaction time was 48 h. Compound **7a** was obtained as a clear oil: yield 1.5 g (53%); ^{1}H NMR (CD_3CN) δ 4.10 (s, 4 H), 3.53 (m, 4 H), 3.43 (t, J = 7.1 Hz, 2 H), 2.20–2.40 (m, 6 H), 2.0 (m, 2 H); MS, *m/e* (relative intensity) 136 (10.3), 98 (28.5), 88 (44.5), 85 (22.4). Anal. ($C_{12}H_{18}ClNO_5S$) C, H, N; C: calcd, 44.5; found, 43.9.

***N*-(4-Bromo-2-butynyl)succinimide (5b).** *N*-[4-(Diethylamino)-2-butynyl]succinimide (**4b**) was prepared as described previously.[20] A solution of **4b** (28 g, 130 mmol) in $CHCl_3$ was added dropwise to 14.7 g (140 mmol) of BrCN in $CHCl_3$. The solution was stirred at room temperature overnight. The organic

(9) Ringdahl, B.; Roch, M.; Jenden, D. J. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 464.
(10) Cohen, S. G.; Elkind, J. L.; Chishti, S. B.; Giner, J.-L. P.; Reese, H.; Cohen, J. B. *J. Med. Chem.* **1984**, *27*, 1643.
(11) Höltje, H.-D.; Jensen, B.; Lambrecht, G. *Eur. J. Med. Chem.—Chim. Ther.* **1978**, *13*, 453.
(12) Mutschler, E.; Höltje, H.-D.; Lambrecht, G.; Moser, U. *Arzneim.-Forsch./Drug Res.* **1983**, *3*, 806.
(13) Barrett, A. N.; Roberts, G. C. K.; Burgen, A. S. V.; Clore, G. M. *Mol. Pharmacol.* **1983**, *24*, 443.
(14) Ing, H. R.; Kordik, P.; Tudor Williams, D. P. H. *Br. J. Pharmacol.* **1952**, *7*, 103.
(15) Birdsall, N. J. M.; Burgen, A. S. V.; Hulme, E. C. *Mol. Pharmacol.* **1978**, *14*, 723.
(16) Ringdahl, B. *Eur. J. Pharmacol.* **1984**, *99*, 177.
(17) Ehlert, F. J.; Jenden, D. J. *Mol. Pharmacol.* **1985**, *28*, 107.
(18) Ross, S. B.; Fröden, O. *Eur. J. Pharmacol.* **1970**, *13*, 46.
(19) Resul, B.; Lewander, T.; Zetterström, T.; Ringdahl, B.; Muhi-Eldeen, Z.; Dahlbom, R. *J. Pharm. Pharmacol.* **1980**, *32*, 439.
(20) Dahlbom, R.; Karlen, B.; George, R.; Jenden, D. J. *J. Med. Chem.* **1966**, *9*, 843.

phase was washed with 0.5 N HCl and water. The solvent was evaporated, and a liquid fraction was removed by vacuum distillation (bp ~40 °C at 0.1 mmHg). The residue was crystallized from ethanol–water to give 19 g (65%) of **5b** as white crystals: mp 85–86 °C; ^{1}H NMR (CD_3CN) δ 4.22 (t, J = 2.1 Hz, 2 H), 3.99 (t, J = 2.1 Hz, 2 H), 2.64 (s, 4 H); MS, m/e (relative intensity) 231 (3.3), 229 (3.6), 150 (100). Anal. ($C_8H_8BrNO_2$) C, H, N.

[4-(2,5-Dioxopyrrolidinyl)-2-butynyl]dimethylsulfonium perchlorate (6b) was prepared from **5b** as described above for **6a**. The yield of **6b** was 59%: mp 114–115 °C (from acetone–ether); ^{1}H NMR (CD_3CN) δ 4.31 (t, J = 2.1 Hz, 2 H), 4.11 (t, J = 2.1 Hz, 2 H), 2.80 (s, 6 H), 2.67 (s, 4 H); MS, m/e (relative intensity) 151 (8.4), 150 (17.9), 113 (8.0), 112 (8.8), 99 (16.1), 84 (17.9). Anal. ($C_{10}H_{14}ClNO_6S$) C, H, N.

[4-(2,5-Dioxopyrrolidinyl)-2-butynyl]thiolanium perchlorate (7b) was prepared similarly from **5b**, tetrahydrothiophene, and $AgClO_4$. The reaction time was 48 h. Compound **7b** was obtained in 73% yield: mp 127–129 °C (from acetone–ether); ^{1}H NMR (CD_3CN) δ 4.25 (t, J = 2.1 Hz, 2 H), 4.07 (t, J = 2.1 Hz, 2 H), 3.35–3.65 (m, 4 H), 2.67 (s, 4 H), 2.15–2.45 (m, 4 H); MS, m/e (relative intensity) 151 (6.2), 150 (10.3), 113 (6.5), 112 (8.4), 99 (10.4), 88 (16.0), 84 (22.2). Anal. ($C_{12}H_{16}ClNO_6S$) C, H, N.

Guinea Pig Ileum and Urinary Bladder. Segments of the ileum[21] and strips of the bladder[22] were set up in Tyrode solution (pH 7.4) at 37 °C as described previously. The Tyrode solution contained hexamethonium (0.3 mM). Contractions were recorded isotonically at 1 g of tension by using an electromechanical displacement transducer and a potentiometric recorder. Concentration–response curves were constructed by the cumulative dose–response technique by increasing stepwise the concentration of agonist by a factor of 2.15 (ileum) or 3.16 (bladder).

The dissociation constant K_D and the efficacy (relative to that of carbachol) of **6a** were estimated after fractional receptor inactivation with propylbenzilylcholine mustard according to a previously described method.[21,22] Propylbenzilylcholine mustard was applied for 15 min at a concentration of 0.5 μM (ileum) or 0.1 μM (bladder). The K_D values and relative efficacies of **6b**, **7a**, and **7b** were determined by comparison of their concentration–response curves with that of carbachol.[21,22] The dissociation constant of **7b** at receptors in the bladder was estimated as described by Arunlakshana and Schild[23] with carbachol as the agonist. Compound **7b** was allowed to equilibrate with the bladder strips for 20 min before the addition of carbachol.

Frog Rectus Abdominis. A standard frog rectus abdominis preparation was set up at 22 °C in aerated Clark–Ringer solution (pH 7.4) as described previously.[16] Contractions were recorded as described above for the ileum. The preparation was exposed to each drug concentration for 5 min. Concentration–response curves were recorded noncumulatively. Equipotent molar ratios relative to carbachol were determined in three-point assays.[24]

Salivation and Acute Toxicity. Male Swiss–Webster mice weighing 24–30 g were used. Drugs were dissolved in 0.9% NaCl and administered iv in the tail. ED_{50} values for salivation and LD_{50} values were estimated by the up-and-down method as described previously,[3] with six or more animals being used for each determination.

Muscarinic Receptor Binding Assay. Cerebral cortex from male Sprague–Dawley rats (200–300 g body weight) was homogenized in 50 volumes of 50 mM phosphate buffer (81 mM Na^+, 9.1 mM K^+, 50 mM PO_4; pH 7.4). The homogenate was centrifuged at 30000g for 10 min and resuspended in phosphate buffer to a concentration of 10 mg of original wet tissue weight/mL of buffer. The binding of (–)-[^{3}H]NMS (87 Ci/mmol) was measured by the filtration assay of Yamamura and Snyder.[25] Homogenate of cortex (0.1 mL) was incubated with nonlabeled ligand and (–)-[^{3}H]NMS (0.3 nM) in a total volume of 2 mL of 50 mM phosphate buffer. Incubations lasted for 30 min at 30 °C. Binding in the presence of 10 μM atropine was defined as nonspecific.

The IC_{50} values (concentration that causes half-maximal inhibition of specific (–)-[^{3}H]NMS binding) of nonlabeled ligands were corrected for receptor occupancy by (–)-[^{3}H]NMS as described by Cheng and Prusoff.[26] Additional binding parameters were determined by nonlinear least-squares regression analysis. A two-site equation was fitted to the ligand/(–)-[^{3}H]NMS competition data to give apparent dissociation constants at the high (K_H) and low (K_L) affinity binding sites as well as the relative proportions of the two sites.[27] The apparent dissociation constants were corrected for receptor occupancy by (–)-[^{3}H]NMS to give the true high (K_H) and low (K_L) affinity dissociation constants. In some instances the data were better described by a one-site competitive inhibition equation.[27] The dissociation constant of (–)-[^{3}H]NMS (0.070 nM) was determined independently by Scatchard analysis of seven-point (–)-[^{3}H]NMS binding isotherms using a centrifugation assay.[15]

Acknowledgment. This work was supported by U.S. Public Health Service Grants GM37816 and MH17691. Skillful technical assistance by Margareth Roch and excellent secretarial assistance by Holly Batal and Nelly Canaan are gratefully acknowledged.

Registry No. **4b**, 7591-19-7; **5a**, 85733-62-6; **5b**, 110826-55-6; **6a**, 110826-52-3; **6b**, 110826-57-8; **7a**, 110826-54-5; **7b**, 110826-59-0; dimethyl sulfide, 75-18-3; tetrahydrothiophene, 110-01-0.

(21) Ringdahl, B. *J. Pharmacol. Exp. Ther.* **1984**, *229*, 199.
(22) Ringdahl, B.; Markowicz, M. E. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 789.
(23) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.
(24) Edinburgh Pharmacology Department Staff *Pharmacological Experiments on Isolated Preparations*; Livingstone: Edinburgh, 1968; pp 13, 28.
(25) Yamamura, H. I.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1725.
(26) Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
(27) Birdsall, N. J. M.; Burgen, A. S. V.; Hulme, E. C. *Recent Advances in Receptor Chemistry*; Gualtieri, F., Giannella, M., Melchiorre, C., Eds.; Elsevier/North-Holland Biomedical: Amsterdam, 1979; p 71.