A major thrust in future research will be to further explore and define the shape commonality index.

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Registry No. 1, 105763-92-6; 2, 105763-93-7; 3, 105763-94-8; 4, 95333-69-0; 5, 105763-95-9; 6, 105763-96-0; 7, 95333-70-3; 8, 105763-97-1; 9, 105763-98-2; 10, 105763-99-3; 11, 95333-91-8; 12, 95333-90-7; 13, 105764-00-9; 14, 105764-01-0; 15, 95333-88-3; 16, 105764-02-1; 17, 95333-53-2; 18, 105764-03-2; 19, 95333-51-0; 20, 101913-05-7; 21, 95460-09-6; 22, 95333-54-3; 23, 95333-50-9; 24, 95333-61-2; 25, 95333-79-2; 26, 95333-71-4; 27, 95333-72-5; 28, 95333-59-8; 29, 95333-62-3; 30, 105764-04-3; 31, 95333-73-6; 32, 95333-52-1; 33, 95333-74-7; 34, 105764-05-4; 35, 104197-16-2; 36, 23269-10-5; 37, 95333-58-7; 38, 95333-75-0; 39, 95333-76-9; 40, 95333-48-5; 41, 95333-77-0; 42, 95333-80-5; 43, 95333-81-6; 44, 95333-64-5; 45, 95333-78-1; 46, 95333-66-7; 47, 95333-78-1; 48, 95333-68-9; 49, 95333-49-6; 50, 985333-81-6; 51, 95333-57-6; 52, 95333-60-1; dopamine β-hydroxylase, 9013-38-1.

Tertiary 2-Haloethylamine Derivatives of the Muscarinic Agent McN-A-343, [4-[[*N*-(3-Chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium Chloride

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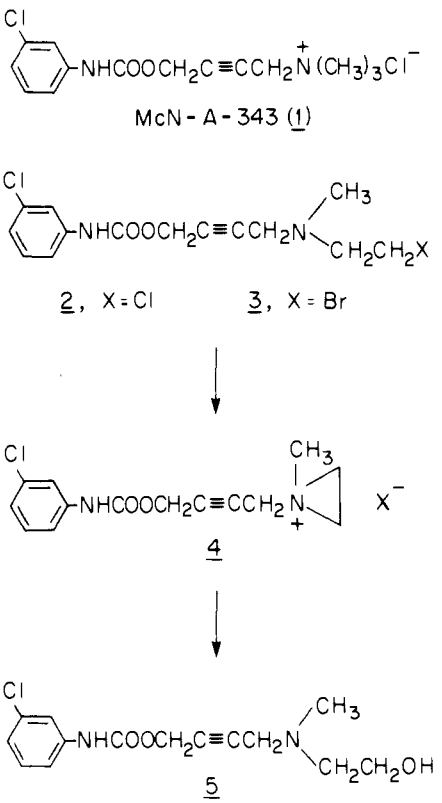
4-[(2-Chloroethyl)methylamino]-2-butynyl *N*-(3-chlorophenyl)carbamate (**2**) and 4-[(2-bromoethyl)methylamino]-2-butynyl *N*-(3-chlorophenyl)carbamate (**3**) were synthesized. Compounds **2** and **3** cyclized at neutral pH to an aziridinium ion (**4**). The rate constants for the cyclization of **2** and **3** at 37 °C were about 0.01 and 0.4 min^{-1}, respectively, as measured by titrimetric analysis and by ^1H NMR spectroscopy. The aziridinium ion had $^1/_4$ the potency of McN-A-343 (**1**) as a ganglionic muscarinic stimulant in the anesthetized, pentolinium-treated rat but showed no muscarinic effects on the isolated guinea pig ileum. It caused alkylation of muscarinic receptors in homogenates of the rat cerebral cortex. An irreversible blockade of central muscarinic receptors was also observed after intravenous administration of **3** to mice. Because of its selectivity, irreversible actions, and ability to pass into the central nervous system, **3** should become a valuable tool in studies of muscarinic receptors.

McN-A-343, **1** (Scheme I), was first described by Roszkowski.[1] Like many other muscarinic agents, such as muscarine and oxotremorine, **1** is potent in stimulating muscarinic receptors in autonomic ganglia. The unique feature of **1** is its relatively weak muscarinic actions outside ganglia, as for example in the isolated heart and on intestinal smooth muscle.[1,2] Many analogues of **1** have been synthesized, but only a few of these possess selectivity and potency similar to that of **1**.[3-7] In particular, no close analogues of **1** capable of passing into the central nervous system are presently known. Since ganglionic muscarinic receptors appear to resemble certain central muscarinic receptors (M1 receptors) in their structural specificity,[8] such analogues have been suggested as potential therapeutic agents in conditions associated with deficits in central cholinergic function, e.g. Alzheimer's disease.[9]

We describe here an *N*-methyl-*N*-(2-chloroethyl)amino derivative (**2**, BR 383) and an *N*-methyl-*N*-(2-bromoethyl)amino derivative (**3**, BR 384) which are capable of cyclizing to an aziridinium ion (**4**) closely resembling **1**. This aziridinium ion maintains the selectivity shown by **1** for ganglionic muscarinic receptors. We also show that **3** enters into the central nervous system after systemic administration and that **4** interacts covalently with central muscarinic receptors.

Scheme I



Results

Synthesis of 2 and 3. Amino alcohol **5** was obtained in a Mannich reaction from 2-propynyl *N*-(3-chlorophenyl)carbamate, paraformaldehyde, and *N*-methyl-*N*-(2-hydroxyethyl)amine. 2-Chloroethylamine **2** was pre-

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Table I. Rate Constants and Half-Lives of **2** and **3** in CD_3CN–Deuterated Phosphate Buffer (pH 7.4) at 37 °C[a]

(2-haloethyl)amine $\xrightarrow{k_1}$ aziridinium ion $\xrightarrow{k_2}$ hydrolysis products

compd	k_1,[b] min^{-1} ($t_{1/2}$, min)		k_2,[c] min^{-1} ($t_{1/2}$, min)
	from disappearance of **2** or **3**	from formation of **4**	
2	0.0089 ± 0.0001 (112)		
3	0.27 ± 0.02 (3.7)	0.24 ± 0.01 (4.2)	0.099 ± 0.012 (10.1)

[a] Values are means ± standard errors from three independent experiments. [b] Apparent first-order rate constant for the cyclization reaction. [c] Rate constant for the decay of the aziridinium ion.

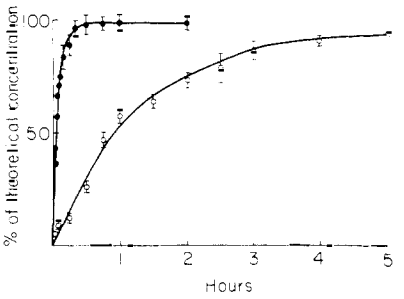

Figure 1. Chloride ion release (○) from **2** and bromide ion release (●) from **3** in 50 mM phosphate buffer (pH 7.4) at 37 °C. The ordinate shows concentrations of halide ion as a percentage of the maximum of 1 equiv/mol of the parent compounds. Values are means ± standard errors from three determinations. The curves are the best fit to a first-order rate equation.

pared by treatment of **5** with thionyl chloride in dichloromethane. Treatment of **5** with triphenylphosphine and carbon tetrabromide in dichloromethane yielded 2-bromoethylamine **3**.

Kinetics of Solvolysis of 2 and 3. Rates of cyclization of **2** and **3** were measured from halide ion release at 37 °C. Because of the low solubility of the free bases of **2** and **3** in neutral aqueous solution, the cyclization experiments were performed in 50 mM phosphate buffer (pH 7.4) containing 20% acetone (by volume). Under these conditions, **2** and **3** released 1 equiv of chloride and bromide ion, respectively. The cyclization reactions followed first-order kinetics (Figure 1). The apparent first-order rate constants for cyclization of **2** and **3**, respectively, were 0.012 ± 0.001 and 0.46 ± 0.05 min^{-1} as estimated by nonlinear-regression analysis.

The rates of cyclization of **2** and **3** and the rates of formation and hydrolysis of **4** were also studied by ^1H NMR. The oxalate salts of **2** and **3** were dissolved in CD_3CN–deuterated phosphate buffer (2:3 by volume) to liberate the free bases. Spectra were then recorded at various times during a 3-h period (**3**) and an 8-h period (**2**). Under these conditions, the *N*-methyl signal of **3** appeared as a singlet at δ 2.6. The $\equiv CCH_2N$ protons (apparent singlet at δ 3.7) and the CH_2Br protons (triplet at δ 3.6) also were well-separated from other peaks. The intensity

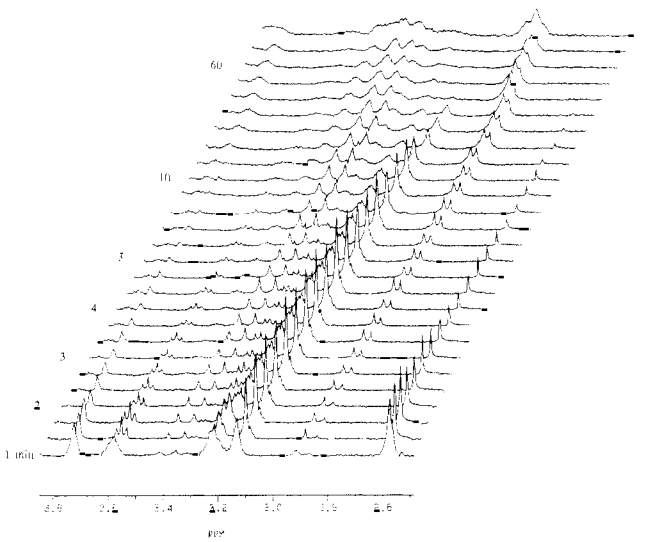

Figure 2. Superimposed ^1H NMR spectra (360 MHz) of **3** at various times after its dissolution in CD_3CN–deuterated phosphate buffer (pH 7.4) at 37 °C.

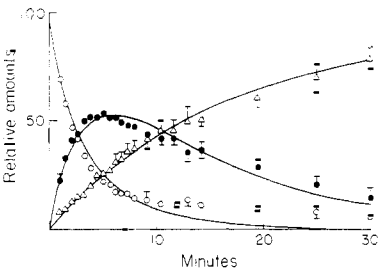

Figure 3. Relative amounts of **3** (○), **4** (●), and their hydrolysis products (△) at various times after dissolution of **3** in CD_3CN–deuterated phosphate buffer (pH 7.4) at 37 °C. Relative amounts were obtained by ^1H NMR measurements. The amount of **3** at 0 time equals 100. The curves are the best fit to the relevant rate equations.

of these signals clearly decreased with time after dissolution as can be seen from Figure 2, where the spectra are displayed in a two-dimensional, stacked format. Aziridinium ion **4** was already present in appreciable amounts 1 min after dissolution of **3** as evidenced by its *N*-methyl signal (singlet at δ 3.1). The intensity of this signal increased initially but decreased as the reaction progressed. A signal at δ ~2.9 increased steadily with time and was assigned to the *N*-methyl group of amino alcohol **5** or of its phosphate ester. Such phosphate esters have been shown previously to be formed from aziridinium ion hydrolysis in phosphate buffer.[10,11] These spectral assignments are in general agreement with those reported previously for some [*N*-(2-haloalkyl)amino]tetraline derivatives.[12]

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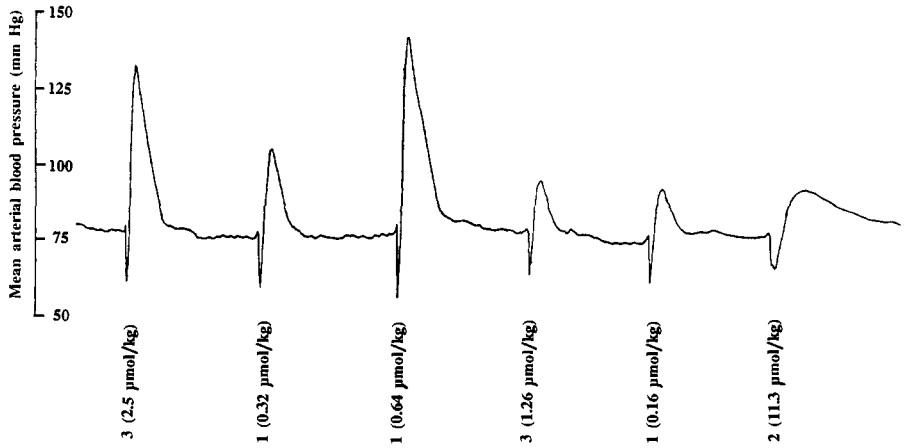

Figure 4. Changes in mean arterial blood pressure in the anesthetized, pentolinium-treated rat caused by 1–3 administered intravenously. Compounds 2 and 3 were cyclized before injection as described in the Experimental Section. Doses of 2 and 3 refer to the parent compounds.

Relative amounts of 3–5 at various times were obtained by integration over their *N*-methyl signals. The results are summarized in Figure 3. Assuming that 3 was converted quantitatively to 5 (or its phosphate ester) via the aziridinium ion 4, we estimated rate constants for the formation and decay of 4. Nonlinear-regression analysis as described in the Experimental Section yielded rate constants (k_1) for the disappearance of 3 and for the formation of 4 that were in excellent agreement (Table I). The rate constant (k_2) for the decay of 4 (Table I) and that for the formation of the hydrolysis products (0.060 ± 0.007 min^{-1}) also were similar in magnitude. From the values of k_1 and k_2, it may be calculated (see eq 2 and 3 of the Experimental Section) that the peak aziridinium ion concentration was 51% of the initial concentration of 3 at 6.3 min after dissolution, in excellent agreement with the experimental results (Figure 3).

A similar analysis was performed with 2. The rate constant (k_1) for the disappearance of 2, as estimated from the decline of its *N*-methyl signal, is given in Table I. This rate constant was about 10-fold smaller than the rate constant for the decay of the aziridinium ion (4) estimated as above. As a result, the concentrations of 4 were quite low. Thus accurate measurements of its formation and decay were difficult to obtain. However, it may be calculated that the peak aziridinium ion concentration (11%) occurred 27 min after dissolution of 2. The rate constant for the formation of hydrolysis products (0.0080 ± 0.0005 min^{-1}) was similar to k_1 (Table I).

Muscarinic Actions. Compounds 2 and 3, precyclized to provide maximal concentration of the aziridinium ion, behaved similarly to 1 in being unable to cause contractions of the isolated guinea pig ileum when tested at concentrations up to 0.1 mM.

When injected intravenously to mice, compounds 1–3 produced no visible central or peripheral muscarinic effects such as tremor or salivation. The LD_{50} values (iv) of 1, 2, and 3 in mice were 3.7 ± 0.5, 166 ± 23, and 57.2 ± 7.9 μmol/kg, respectively.

Changes in mean arterial blood pressure of the anesthetized rat treated with the nicotinic blocker pentolinium were used as a measure of ganglionic muscarinic activity. The initial blood pressure in this preparation was 72 ± 3 mmHg and the initial heart rate was 270 ± 10 beats/min. Compound 3 after cyclization produced a dose-dependent increase in blood pressure. This pressor response was preceded by a short-lasting decrease in blood pressure. These effects were qualitatively similar to those elicited by 1 (Figure 4). Assuming that the peak concentration of 4 was 51% of the initial concentration of 3 (see above), it was estimated that 4 had about $^1/_4$ the potency of 1. The pressor response to 2.5 μmol/kg of 3 (1.3 μmol/kg of 4) was abolished by *N*-methylatropine (0.1 μmol/kg). Compound 2 produced longer lasting pressor and depressor responses than did 1 and 3 (Figure 4). The maximum pressor response obtainable with 2 was lower than those produced by 1 and 3, which appeared to give similar maximum response. Amino alcohol 5 (16 μmol/kg) elicited a depressor response only. Compound 3, similarly to 1, produced a dose-dependent increase in heart rate. At a dose of 2.5 μmol/kg (1.3 μmol/kg of 4), this increase was 127 ± 5 beats/min. A similar increase (102 ± 10 beats/min) was observed with 0.3 μmol/kg of 1. These increases in heart rate were abolished by *N*-methylatropine (0.1 μmol/kg).

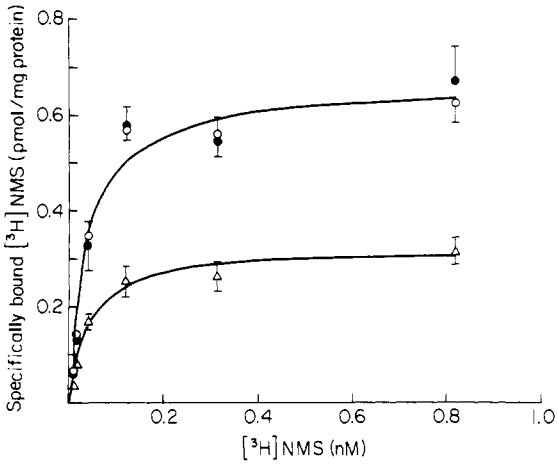

Figure 5. Effect of 3 on the binding of [^{3}H]NMS to homogenates of rat cerebral cortex. Specific [^{3}H]NMS binding was measured in control homogenates (○) and homogenates which had been incubated at 37 °C with 3 (1.0 μM) (△) and 3 (1.0 μM) plus atropine (1.0 μM) (●). The data points represent the mean binding values ± standard error of three experiments, each done in triplicate.

Alkylation of Muscarinic Receptors. Incubation of homogenates of the rat cerebral cortex with precyclized 3 caused an irreversible inhibition of the binding of (−)-[^{3}H]-*N*-methylscopolamine ([^{3}H]NMS). Figure 5 shows measurements of [^{3}H]NMS binding in cortical homogenates which had been incubated in the presence and absence

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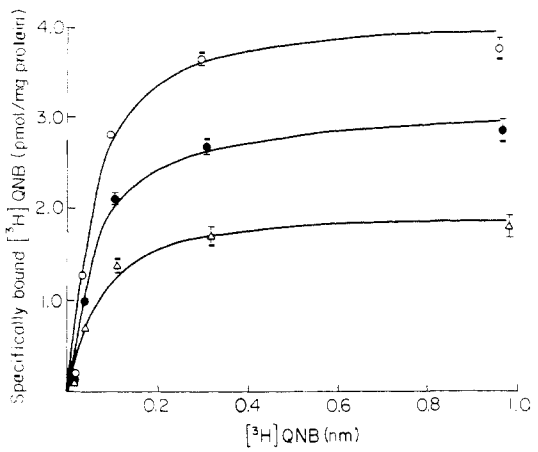

Figure 6. Effect of treatment with **3** in vivo on [^{3}H]QNB binding to mouse forebrain. Specific [^{3}H]QNB binding was measured in homogenates of the forebrain of saline treated (○), **3** treated (50 μmol/kg) (△), and **3** (50 μmol/kg) plus atropine treated (5.0 μmol/kg) (●) mice. The data points are the mean binding values ± standard error of five experiments, each done in triplicate.

of the aziridinium ion as described in the Experimental Section. Nonlinear-regression analysis of the control binding measurements showed that the data were consistent with a simple one-site model having a dissociation constant of 0.044 nM and a binding capacity of 0.68 pmol/mg of protein. The binding capacity of [^{3}H]NMS was reduced by 51% following incubation of the cortex with the aziridinium ion at a concentration equivalent to that derived from a 1.0 μM solution of the parent mustard. Nonlinear regression and analysis of variance showed a significant increase in residual error when the data from the control and **3**-treated homogenates were fitted simultaneously sharing the estimate of the binding capacity between them ($F_{1,8}$ = 107.4; $P = 6 \times 10^{-6}$). In contrast, there was no significant increase in residual error when the data were fitted simultaneously sharing the estimate of the control dissociation constant between them ($F_{1,8}$ = 0.233; P = 0.64). These results indicate that incubation with **3** caused a significant reduction in binding capacity but no significant change in the dissociation constant of [^{3}H]NMS. It can also be seen in Figure 5 that the reduction in the binding capacity caused by **3** was prevented by atropine (1.0 μM).

An irreversible blockade of muscarinic receptors also occurred following injection of **3** into mice. Figure 6 shows measurements of (−)-[^{3}H]-3-quinuclidinyl benzilate ([^{3}H]QNB) binding in homogenates of the forebrains of mice that had been injected with **3** 4.3 h earlier. Nonlinear-regression analysis of the control binding data according to a simple one-site model provided estimates of 0.077 nM and 4.37 pmol/mg of protein for the dissociation constant and binding capacity of [^{3}H]QNB, respectively. Treatment of mice with **3** (50 μmol/kg) caused a 52% reduction in the binding capacity of [^{3}H]QNB (2.10 pmol/mg of protein) and a small change in the dissociation constant (0.087 nM). Nonlinear regression and analysis of variance as described above showed that this decrease in binding capacity was significant ($F_{1,8}$ = 46.21; $P = 1.4 \times 10^{-4}$) whereas the change in the dissociation constant was not significant ($F_{1,8}$ = 0.137; P = 0.72). Premedication of mice with atropine (5.0 μmol/kg) 15 min before injection with **3** partially prevented the reduction in the binding capacity caused by treatment with **3**. In mice treated with both atropine and **3**, the dissociation constant and binding capacity of [^{3}H]QNB were 0.086 nM and 3.30 pmol/mg of protein, respectively.

Treatment with **3** also caused a significant reduction in the binding of [^{3}H]QNB at a single concentration (0.34 nM) in the hindbrain of the mice described above. Measurements of [^{3}H]QNB binding in control, **3**-treated, and **3**-plus-atropine-treated mice were 0.277 ± 0.028, 0.128 ± 0.01, and 0.206 ± 0.023 pmol/mg of protein, respectively.

Discussion

The basic assumption underlying our ^{1}H NMR evaluation of the rate constants for the formation (k_1) and decay (k_2) of **4** was that the sum of the concentrations of **3**–**5** and the phosphate ester of **5** at any time equaled the initial concentration of **3**. This assumption appeared justified at least in the early stages of the solvolysis of **3** since no indication of additional reaction products was found in the ^{1}H NMR spectra. Also, the rate constants for the cyclization of **2** and **3** as estimated from halide ion release (Figure 1) agreed reasonably well with those obtained from ^{1}H NMR measurements of the disappearance of **2** and **3**. In agreement with previous results obtained with (2-haloethyl)amines related to oxotremorine,[13-15] 2-bromoethylamine **3** cyclized about 35-fold more rapidly than 2-chloroethylamine **2**. The rate constant for the decay of aziridinium ion **4** was similar to those observed for aziridinium ions related to oxotremorine.[13-15] The observation that the rate constant for the cyclization of **2** was about 10-fold lower than the rate constant for the decay of the aziridinium ion readily explains the low peak levels of the ion when formed from **2**.

Compounds **2** and **3** when injected into mice did not cause the typical muscarinic effects (e.g. tremor and salivation) seen after administration of tertiary muscarinic agents such as oxotremorine.[16] They resembled **1** in being unable to stimulate contractions of the guinea pig ileum at relevant concentrations. After cyclization to aziridinium ion **4**, they elicited a pressor response and an increase in heart rate in the anesthetized rat treated with pentolinium. This preparation, which resembles that with the pithed rat[17] in its sensitivity to **1**, responded to oxotremorine with a predominantly depressor response. Since the parent compounds (**2** and **3**) and alcohol **5** gave no increase in blood pressure, the pressor response observed appeared to be associated entirely with the aziridinium ion (**4**). Furthermore, since the effects of **4** were abolished by *N*-methylatropine, they most likely were mediated by activation of muscarinic receptors. Collectively, our results indicate that **4** caused its cardiovascular effects by a mechanism analogous to that suggested for **1**, i.e. by selective stimulation of ganglionic muscarinic receptors leading to the release of norepinephrine at the nerve terminals.[1]

The aziridinium ion caused a reduction in the binding capacity of [^{3}H]NMS in the rat cerebral cortex without significantly affecting the apparent affinity of [^{3}H]NMS. This inhibitory effect persisted after extensive washing and was prevented by atropine (Figure 5). Such behavior is consistent with a covalent interaction of **4** with muscarinic receptors. Although **3** produced no visible central muscarinic effects in mice, it was capable of entering into the central nervous system. Thus mice injected intravenously

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with **3** showed a long-lasting reduction in the number of muscarinic binding sites in the brain. This observation can be best explained by the presence of **4** in the brain after systemic administration of **3**. Because of its selectivity, irreversible actions, and ability to pass into the brain, **3** should become a valuable tool in investigations of muscarinic receptor subtypes and their function.

Experimental Section

Melting points were determined with a heated metal block using glass capillaries and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and agreed with theoretical values to within ±0.4%. Mass spectra were recorded on a Hewlett-Packard 5981A mass spectrometer at 70 eV. ^{1}H NMR spectra were obtained at 23 °C (37 °C for the kinetic experiments) on a Bruker AM360/Wb spectrometer at 360 MHz. Chemical shifts are reported in ppm (δ) downfield from internal $(CH_3)_4Si$ standard.

4-[(2-Hydroxyethyl)methylamino]-2-butynyl *N*-(3-Chlorophenyl)carbamate (5). 2-Propynyl *N*-(3-chlorophenyl)carbamate was synthesized from 3-chlorophenyl isocyanate and propargyl alcohol as described previously.[18] A mixture of 2-propynyl *N*-(3-chlorophenyl)carbamate (39 g, 0.19 mol), *N*-methyl-*N*-(2-hydroxyethyl)amine (14 g, 0.19 mol), paraformaldehyde (6.1 g, 0.20 mol), and CuCl (0.4 g) in 300 mL of dioxane was heated at 80 °C for 3 h. The dioxane was evaporated in vacuum and the residue was dissolved in 0.1 N HCl. The aqueous solution was extracted twice with ether, and K_2CO_3 and NH_4OH were added to adjust the pH to 8. Extraction with CH_2Cl_2 (3 × 100 mL), drying (K_2CO_3), and evaporation yielded crude **5**, which was recrystallized from ether–pentane: mp 81–82 °C; yield 39 g (69%); ^{1}H NMR (CD_3CN) δ 7.99 (br, 1 H), 7.56 (m, 1 H), 7.31 (m, 2 H), 7.08 (m, 1 H), 4.77 (t, 2 H, J = 2.0 Hz), 3.52 (t, 2 H, J = 5.9 Hz), 3.38 (s, 2 H), 2.65 (br, 1 H), 2.51 (t, 2 H, J = 5.9 Hz), 2.27 (s, 3 H). The oxalate salt was prepared by the addition of oxalic acid in ether to a solution of **5** in ether: mp 121–122 °C (from ethanol–ether); MS m/e (relative intensity) 267 [M^+ + 2 − CH_3OH] (13), 265 [M^+ − CH_3OH] (36), 222 (25), 69 (100). Anal. ($C_{16}H_{19}ClN_2O_7$) C, H, N.

4-[(2-Chloroethyl)methylamino]-2-butynyl *N*-(3-Chlorophenyl)carbamate (2) Oxalate. Compound **5** (3.0 g, 10 mmol) was dissolved in anhydrous CH_2Cl_2 (50 mL), and $SOCl_2$ (1.6 g, 13 mmol) in CH_2Cl_2 (20 mL) was added dropwise at 0 °C. The solution was refluxed for 3 h and concentrated under vacuum. The residue was dissolved in an ice-cold water–ether mixture and K_2CO_3 was added under vigorous stirring until the aqueous phase became alkaline. The ethereal layer was separated and dried breifly over Na_2SO_4. The oxalate salt was precipitated by the addition of oxalic acid in ether: mp 115–116 °C (from ethanol–ether), yield 2.2 g (54%); ^{1}H NMR (CD_3OD) δ 7.59 (m, 1 H), 7.28 (m, 2 H), 7.04 (m, 1 H), 4.86 (s, 2 H), 4.10 (s, 2 H), 3.90 (t, 2 H, J = 6 Hz), 3.49 (t, 2 H, J = 6 Hz), 2.89 (s, 3 H); MS m/e (relative intensity) 267 [M^+ + 2 − CH_3Cl] (14), 265 [M^+ − CH_3Cl] (44), 222 (26), 69 (100). Anal. ($C_{16}H_{18}Cl_2N_2O_6$) C, H, N.

4-[(2-Bromoethyl)methylamino]-2-butynyl *N*-(3-Chlorophenyl)carbamate (3) Oxalate. Compound **5** (5.0 g, 17 mmol) and Ph_3P (5.0 g, 19 mmol) were dissolved in anhydrous benzene, which was then evaporated under vacuum. The residue was dissolved in anhydrous CH_2Cl_2 (100 mL) and the solution was cooled to 0 °C in a sealed bottle flushed with dry nitrogen. Then CBr_4 (7.8 g, 23.5 mmol) dissolved in CH_2Cl_2 (20 mL) was added dropwise through a septum. The mixture was allowed to warm up to room temperature and pentane (200 mL) was added. After filtration, the solvents were removed, and the residue was dissolved in 2 N HCl. The aqueous phase was extracted with ether (3 × 100 mL) and cooled with ice, and ether was added. K_2CO_3 was added to the vigorously stirred mixture to adjust the pH to 8. The organic phase was separated and filtered through Na_2SO_4 into a solution of oxalic acid in anhydrous ether: yield 3.0 g (40%); ^{1}H NMR (CD_3OD) δ 7.60 (m, 1 H), 7.26 (m, 2 H), 7.03 (m, 1 H), 4.85 (s, 2 H), 4.05 (s, 2 H), 3.69 (t, 2 H, J = 6.8 Hz), 3.50 (t, 2 H, J = 6.8 Hz), 2.84 (s, 3 H); MS m/e (relative intensity) 267 [M^+ + 2 − CH_3Br] (32), 265 [M^+ − CH_3Br] (100), 222 (39). Anal. ($C_{16}H_{18}BrClN_2O_6$) C, H, N.

Measurement of Formation and Decomposition of Aziridinium Ion. Halide ion release from **2** and **3** was measured at 37 °C in 50 mM sodium phosphate buffer (pH 7.4)–acetone (4:1 by volume) by argentometric titration as described previously.[13,14] Rate constants for the cyclization were evaluated by fitting a first-order rate equation to the halide release data by nonlinear-regression analysis using a BMDP program (BMDP Statistical Software Inc.).

For the ^{1}H NMR studies of reaction kinetics, the oxalate salts of **2** and **3** (4 mg) were dissolved in CD_3CN (0.2 mL) in an NMR tube, and 0.3 mL of 50 mM NaD_2PO_4–Na_2DPO_4 buffer (pH 7.4) was added. The NMR tube was inserted into the probe at 37 °C and the first spectrum was obtained 1 min after mixing. Spectra were then recorded at 20 s (**3**) or 1 min (**2**) intervals during the early stages of the reaction and at longer time intervals as the experiment proceeded. Rate constants for the cyclization of **2** and **3** and for the formation of **5** were obtained from a first-order rate equation as described above.

The concentration of the aziridinium ion (Q) as a function of time (t) was fitted by nonlinear-regression analysis, using a BMDP program, to eq 1, where Q_0 is the starting concentration of (haloethyl)amine and k_1 and k_2 are the rate constants for the formation and decay of the aziridinium ion, respectively. This analysis provided estimates of k_1 and k_2. At the time (t_{max}) of peak aziridinium ion concentration (Q_{max}), the rates of formation and decomposition of the ion are equal ($dQ/dt = 0$). It follows from eq 1 that

$$Q = \frac{Q_0 k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad (1)$$

$$t_{max} = (\ln k_1 - \ln k_2)/(k_1 - k_2) \quad (2)$$

$$Q_{max} = Q_0 (k_1/k_2)^{k_2/(k_2 - k_1)} \quad (3)$$

Acute Toxicity. LD_{50} values were determined in male Swiss–Webster mice (24–30-g body weight) by intravenous injection (tail vein) by using the up-and-down method.[19] Mortality counts were taken at 30 min. For these experiments, the oxalate salts of **2** and **3** were dissolved in saline and the solutions were kept on ice for no more than 2 h.

Ganglionic Muscarinic Activity. Male Sprague–Dawley rats (250–300-g body weight) were anesthetized with amytal sodium (100 mg/kg, intraperitoneally) and treated with pentolinium tartrate (20 mg/kg, subcutaneously). The trachea was intubated, and the left femoral vein was cannulated for the administration of drugs. Heparin (200 units) was given intravenously. The common carotid artery was then cannulated for measurements of arterial blood pressure and heart rate with a Deseret Transducer System (Deseret Medical Inc.). Body temperature was maintained at 37 °C throughout the experiment. All drugs were dissolved in saline and were administered intravenously in a volume of 0.1 mL. Compounds **2** and **3** were incubated in acetonitrile–phosphate buffer at 37 °C for 30 and 5 min, respectively, to allow formation of the aziridinium ion. The acetonitrile was evaporated before drug dilution. Pretreatment with *N*-methylatropine was carried out 20 min before administration of agonists.

Radioligand Binding. Radioligand binding assays were carried out on homogenates of the rat cerebral cortex, which had been previously incubated in vitro with **3**. The cerebral cortex from male Sprague–Dawley rats (180–250 g) was homogenized in 10 volumes of 50 mM phosphate buffer (pH 7.4). This homogenate was centrifuged at 30000g for 10 min, and the supernatant was discarded. The pellet was resuspended in 50 mM phosphate buffer and centrifuged again as described above. The final pellet was resuspended to a concentration of 50 mg of tissue (original wet weight) per mL of buffer. An aliquot (4.0 mL) of this homogenate and an aliquot of a solution of **3** which had been incubated at 37 °C for 5 min to allow formation of the aziridinium ion were incubated in a final volume of 10 mL of 50 mM phosphate buffer for 30 min at 37 °C. Control tissue was incubated in the same

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manner except for the addition of 3. Following this incubation, the homogenates were washed three times by centrifugation at 30000g for 10 min followed by resuspension in 10 mL of 50 mM phosphate buffer. For measurement of radioligand binding, the final pellets were resuspended to a concentration of 1.0 mg of tissue (original wet weight) per mL of 50 mM phosphate buffer.

Additional radioligand binding assays were carried out on homogenates of the brains of mice which had been treated in vivo with 3. For these experiments, mice were injected intravenously (tail vein) with the parent mustard 3 at a dose of 50 μmol/kg. The mice were killed 4.3 h later, and the forebrain and hindbrain were separated by a midcollicular transection. The forebrain and hindbrain were homogenized in approximately 30 and 100 volumes, respectively, of 50 mM phosphate buffer. These homogenates were washed three times by centrifugation at 30000g for 10 min followed by resuspension in fresh 50 mM phosphate buffer. For measurement of radioligand binding, the final pellets from the forebrain and hindbrain were resuspended to concentrations of 1.0 and 4.0 mg of tissue (original wet weight) per mL of 50 mM phosphate buffer, respectively.

The binding of the specific muscarinic antagonists (-)-[^{3}H]QNB (33.4 Ci/mmol, New England Nuclear) and (-)-[^{3}H]NMS (84.8 Ci/mmol, New England Nuclear) was measured essentially as described previously.[20] An aliquot (1.0 mL) of brain homogenate was incubated in a final volume of 2.0 mL containing 50 mM phosphate buffer. The incubation with (-)-[^{3}H]QNB was 60 min at 37 °C whereas that with (-)-[^{3}H]NMS was 30 min at 30 °C. Nonspecific binding was defined as the residual binding in the presence of 10 μM atropine. Protein was measured by the method of Lowry et al.[21] using bovine serum albumin as the standard.

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Registry No. 2, 123567-32-8; 2·oxalate, 123567-33-9; 3, 123567-34-0; 3·oxalate, 123567-35-1; 4 (X = Cl), 123567-36-2; 4 (X = Br), 123567-37-3; 5, 123567-38-4; 5·oxalate, 123567-39-5; 2-propynyl *N*-(3-chlorophenyl)carbamate, 3004-45-3; *N*-methyl-*N*-(2-hydroxyethyl)amine, 109-83-1.

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Highly Selective κ-Opioid Analgesics. 3. Synthesis and Structure–Activity Relationships of Novel *N*-[2-(1-Pyrrolidinyl)-4- or -5-substituted-cyclohexyl]arylacetamide Derivatives

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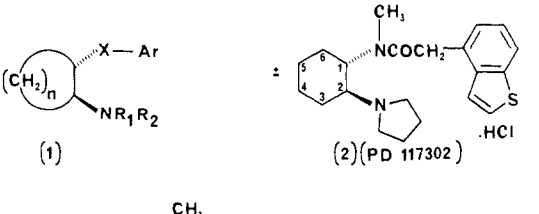
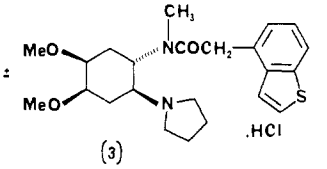
This paper describes the chemical synthesis, μ/κ opioid receptor selectivity and analgesic activity of 14 novel *N*-[2-(1-pyrrolidinyl)-4- or -5-substituted-cyclohexyl]arylacetamide derivatives. The prototype κ-selective agonist, PD117302 (*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzo[*b*]thiophene-4-acetamide, 2) has been regio- and stereoselectively substituted in the C-4 and C-5 positions of the cyclohexyl ring with the methyl ether and spiro tetrahydrofuran groups. It is observed that optimal μ/κ-receptor selectivity is obtained when the oxygen atom of the methyl ether or the tetrahydrofuran ring is joined to the equatorial C-4 position. Hence, (-)-(5β,7β,8α)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzo[*b*]furan-4-acetamide monohydrochloride (21) has exceptionally high κ opioid receptor affinity and selectivity in vitro (κ K_i = 0.83 nM, μ/κ ratio = 1520) is the most potent κ-selective analgesic ever reported. Compound 21 is 25 times more potent than morphine and 17 times more potent than U-62066 (spiradoline, 19) when assayed by the rat paw pressure test by intravenous administration (MPE_{50} = 0.024, 0.6, and 0.4 mg/kg, respectively).

Previous studies[1-9] have established that certain *N*-(2-aminocyclohexyl)arylacetamides with general structure 1 exhibit high in vitro selectivity and affinity for the κ-opioid receptor. These compounds elicit potent analgesia in rodent tests without the undesired μ-opioid effects (respiratory depression, dependence-inducing liability, and inhibition of gastrointestinal motility), which characterize morphine and its congeners.[3]



(1) (2)(PD 117302)



(3)

We have previously shown[1,2] that derivatives of the general structure 1 possess high κ opioid receptor binding affinity and high μ/κ selectivity when the aromatic group

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