

# Differential Blockade of Muscarinic Receptor Subtypes by Polymethylene Tetraamines. Novel Class of Selective Antagonists of Cardiac M-2 Muscarinic Receptors

Carlo Melchiorre,\* Anna Cassinelli, and Wilma Quaglia

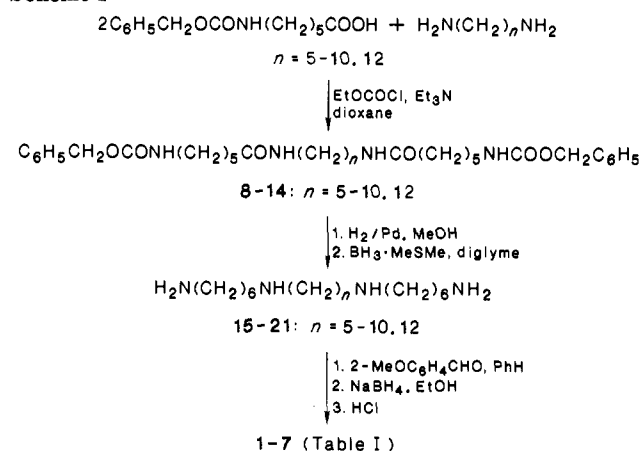
Department of Chemical Sciences, University of Camerino, 62032 Camerino (MC), Italy. Received April 21, 1986

Several *N,N'*-bis[6-[(2-methoxybenzyl)amino]hexyl]-1, $\omega$ -alkanediamine tetrahydrochlorides (1-7) were synthesized and evaluated for their blocking activity on muscarinic receptors in guinea pig atria and rat ileum and bladder. The results were compared with those obtained for the classical nonselective muscarinic antagonist atropine. It was discovered that optimum activity is associated with an eight-carbon chain (compound 4) in guinea pig atria whereas, in both rat ileum and bladder, the 12-carbon analogue 7 had the highest activity. In addition, polymethylene tetraamines 1-6 displayed high selectivity toward guinea pig atria muscarinic receptors. The discriminatory power of 1-6 was not shared by 7. All the tetraamines were shown to be competitive antagonists of muscarinic receptors. *N,N'*-Bis[6-[(2-methoxybenzyl)amino]hexyl]-1,8-octanediamine (4) was the most potent and selective toward muscarinic receptors in atria, with a  $pA_2$  value of 8.13 and a selectivity ratio (atria vs. ileum or bladder) of ca. 270. At a concentration of 10  $\mu$ M, tetraamine 4 did not affect histamine and 5-hydroxytryptamine receptors of guinea pig ileum or  $\alpha$ -adrenoreceptors of guinea pig atria whereas it inhibited postsynaptic  $\alpha$ -adrenoreceptors of rat vas deferens with a  $-\log K$  value of 5.23 and nicotinic receptors of frog rectus abdominis with an  $IC_{50}$  value of 0.23  $\mu$ M. It is concluded that 4 is a novel, powerful, and selective tool in the characterization of muscarinic receptor subtypes.

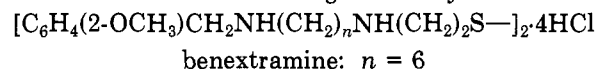
Historically, cholinergic receptors were the first to be divided into subtypes by Dale,<sup>1</sup> that is, muscarinic and nicotinic receptors. Although it was apparent that the muscarinic receptor might not be a homogeneous population because of the extraordinary variety of subserved biological functions, it was only in the late 1970s that the heterogeneity of these receptors was demonstrated, thanks to the discovery of relatively selective ligands.<sup>2</sup> Thus, a division of muscarinic receptors into M-1 and M-2 subtypes was proposed where the M-1 type is located primarily in the central nervous system (CNS), for instance, in the hippocampus and the superior cervical ganglion, while the M-2 type is present mainly in peripheral effector organs, for instance, in atria, smooth muscles, and glands. Pirenzepine was the first selective muscarinic subtype antagonist to display higher affinity toward M-1 rather than M-2 muscarinic receptors.<sup>3</sup> It was also advanced that M-2 muscarinic receptors in peripheral organs are not a homogeneous population since they can be pharmacologically distinguished. For example, gallamine, a neuromuscular blocking agent, has greater affinity for muscarinic receptors in atria than for those in ileum,<sup>4,5</sup> whereas the reverse is true for 4-(diphenylacetoxy)-*N*-methylpiperidine methiodide (4-DAMP).<sup>6,7</sup> However, the selectivity of available ligands is not yet satisfactory, and to characterize and gain further information on muscarinic receptor subtypes, new compounds displaying higher selectivity are needed.

The observation that tetraamine disulfides, a class of irreversible (nonequilibrium in the kinetic sense)  $\alpha$ -adrenoreceptor antagonists, the prototype being benextramine,<sup>8-10</sup> also display significant cholinergic blocking ac-

## Scheme I



tivity<sup>11-14</sup> has prompted us to investigate this property further. To this end we designed the synthesis of 1-7



having the same structural features as benextramine but carrying a different polymethylene chain between the inner nitrogens. These compounds do not bear a disulfide bridge since the all-carbon analogue (2) of benextramine (two methylenes for the disulfide group) behaved in the same way as the parent compound, benextramine, toward muscarinic and nicotinic receptors.<sup>11,12</sup> Furthermore, the chain length separating the inner from the outer nitrogens was kept constant (six methylenes) as in benextramine since changing it from six to five or seven methylenes gave a decrease in both muscarinic and nicotinic activities. In addition, the terminal nitrogens of polymethylene tetraamines 1-7 were substituted with a 2-methoxybenzyl group

- (1) Dale, H. H. *J. Pharmacol. Exp. Ther.* 1914, 6, 147.
- (2) For example, see the following: Birdsall, N. J. M.; Hulme, E. C. *Trends Pharmacol. Sci.* 1983, 4, 459. Hammer, R.; Giachetti, A. *Trends Pharmacol. Sci.* 1984, 5, 18.
- (3) Hammer, R.; Berrie, C. P.; Birdsall, N. J. M.; Burgen, A. S. V.; Hulme, C. P. *Nature (London)* 1980, 283, 90.
- (4) Clark, A. L.; Mitchelson, F. *Br. J. Pharmacol.* 1976, 58, 323.
- (5) Stockton, J. M.; Birdsall, N. J. M.; Burgen, A. S. V.; Hulme, E. C. *Mol. Pharmacol.* 1983, 23, 551.
- (6) Barlow, R. B.; Kitchen, R. *Br. J. Pharmacol.* 1982, 77, 549.
- (7) Barlow, R. B.; Shepherd, M. K. *Br. J. Pharmacol.* 1985, 85, 427.
- (8) Melchiorre, C.; Yong, M. S.; Benfey, B. G.; Brasili, L.; Bolger, G.; Belleau, B. In *Recent Advances in Receptor Chemistry*; Gualtieri, F.; Giannella, M., Melchiorre, C., Eds.; Elsevier/North-Holland Biomedical: Amsterdam, 1979; p 207.

- (9) Melchiorre, C.; Belleau, B. In *Adrenoreceptors and Catecholamine Action*; Kunos, G., Ed.; Wiley: New York, 1981; Part A, p 181.
- (10) Melchiorre, C. *Trends Pharmacol. Sci.* 1981, 2, 209.
- (11) Melchiorre, C.; Yong, M. S.; Benfey, B. G.; Belleau, B. *J. Med. Chem.* 1978, 21, 1126.
- (12) Benfey, B. G.; Yong, M. S.; Belleau, B.; Melchiorre, C. *Can. J. Physiol. Pharmacol.* 1979, 57, 41.
- (13) Benfey, B. G.; Brasili, L.; Melchiorre, C.; Belleau, B. *Can. J. Physiol. Pharmacol.* 1980, 58, 984.
- (14) Benfey, B. G.; Belleau, B.; Brasili, L.; Giannella, M.; Melchiorre, C. *Can. J. Physiol. Pharmacol.* 1980, 58, 1477.

Table I.  $pA_2$  Values for Polymethylene Tetraamines for Muscarinic Receptors in Rat Ileum and Bladder and Guinea Pig Atria

antagonist	n	$pA_2^a$			slope <sup>c</sup>			selectivity ratio <sup>b</sup>		
		atria	ileum	bladder	atria	ileum	bladder	atria/ileum	atria/bladder	ileum/bladder
1	5	6.71 ± 0.15	5.42 ± 0.07	5.21 ± 0.10	0.71 ± 0.06	0.73 ± 0.07	0.67 ± 0.03 <sup>d</sup>	19	32	1.62
2	6	6.87 ± 0.18 <sup>c</sup>	5.29 ± 0.10	5.57 ± 0.31	0.90 ± 0.07	1.07 ± 0.11	0.60 ± 0.04 <sup>d</sup>	38	20	0.52
3	7	7.80 ± 0.08	5.37 ± 0.07	5.63 ± 0.08	1.02 ± 0.03	1.27 ± 0.09	0.90 ± 0.02	269	148	0.55
4	8	8.13 ± 0.21	5.69 ± 0.10	5.70 ± 0.12	1.01 ± 0.08	1.20 ± 0.11	1.10 ± 0.05	275	269	0.98
5	9	7.96 ± 0.11	5.81 ± 0.09	6.08 ± 0.23	1.03 ± 0.09	1.36 ± 0.11	1.08 ± 0.14	141	76	0.54
6	10	7.71 ± 0.20	5.73 ± 0.06	5.49 ± 0.08	1.07 ± 0.13	1.34 ± 0.09	1.06 ± 0.09	95	166	1.74
7	12	7.35 ± 0.09	7.62 ± 0.08	7.13 ± 0.22	1.27 ± 0.10	0.90 ± 0.05	1.08 ± 0.14	0.54	1.66	3.09
atropine		8.69 ± 0.13	8.88 ± 0.06	8.83 ± 0.05	1.08 ± 0.11	1.04 ± 0.03	1.12 ± 0.05	0.64	0.72	1.12

<sup>a</sup> $pA_2$  and slope values  $\pm$  SE of 1–7 and atropine were calculated according to Arunlakshana and Schild.<sup>15</sup>  $pA_2$  is the positive value of the intercept of the line derived by plotting  $\log(DR - 1)$  vs.  $\log$  antagonist. The  $\log(DR - 1)$  was calculated at three different antagonist concentrations, and each concentration was tested at least five times. Dose-ratio (DR) values represent the ratio of the potency of the agonist furtrethonium iodide or muscarine ( $ED_{50}$ ) in the presence of the antagonist and in its absence. Parallelism of dose-response curves was checked by linear regression, and the slopes were tested for significance ( $p < 0.01$ ). <sup>b</sup>The atria/ileum, atria/bladder, and ileum/bladder selectivity ratios are the antilog of the difference between the  $pA_2$  values at atria and ileum, atria and bladder, and ileum and bladder muscarinic receptors, respectively. <sup>c</sup>This value is in agreement with that reported previously by Benfey et al.<sup>12</sup> <sup>d</sup>Significantly different from unity ( $p > 0.01$ ).

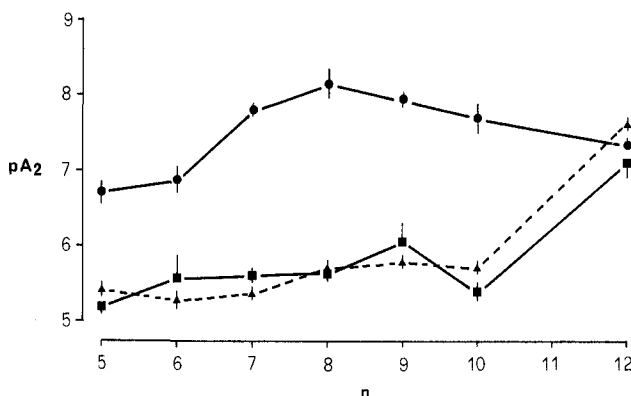


Figure 1. Effect of the carbon chain length of polymethylene tetraamines 1–7 ( $n = 5$ –10, 12) on blocking activity of guinea pig atria (●) and rat ileum (▲) and bladder (■) muscarinic receptors. Data from Table I.

as in benextramine because it has been already observed that its removal yielding an unsubstituted tetraamine disulfide or its substitution with a more polar group, such as a 3,4-dihydroxybenzyl group, produced a decrease in muscarinic blocking activity.<sup>14</sup> Finally, only secondary tetraamines were investigated in this study since N-methylation of benextramine affording a tertiary tetraamine resulted in a decrease in muscarinic blocking activity.<sup>14</sup>

**Chemistry.** The structures of the compounds used in the present study are given in Table I. Since the synthetic pathway reported<sup>11</sup> previously gives 2 in low yields, we looked for a new synthesis that could also be used to prepare analogues 1 and 3–7. This synthetic route is shown in Scheme I. *N*-(Benzyloxycarbonyl)-6-aminocaproic acid was amidated with the appropriate polymethylene diamine ( $n = 5$ –10, 12) to give amides 8–14. The protecting group was removed by hydrogenolysis followed by the reduction of the amide group with borane–methyl sulfide complex to yield unsubstituted tetraamines 15–21. The 2-methoxybenzyl group on the terminal nitrogens was easily introduced by condensation of 15–21 with 2-methoxybenzaldehyde and subsequent reduction of the intermediate Schiff bases to yield tetrahydrochlorides 1–7.

**Pharmacology.** The biological profile of compounds listed in Table I at peripheral muscarinic receptors was

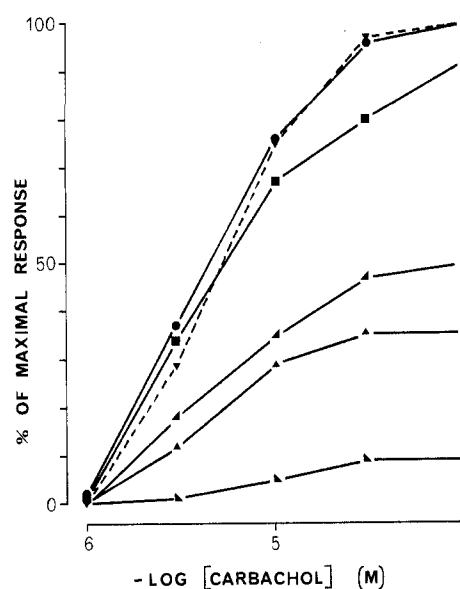


Figure 2. Effects of 4 on noncompetitive occupancy of frog rectus abdominis nicotinic receptors with use of carbachol as agonist; control (●), after exposure to 4 for 30 min: at 0.1  $\mu$ M (■), at 0.3  $\mu$ M (▲) and following 60-min washings (▼), and at 1  $\mu$ M (▲) and following 60-min washings (▲). The results are presented as the mean of five independent observations. SEM were less than 10% and are not shown.

assessed by antagonism of furtrethonium-induced contractions of isolated rat ileum and bladder and by antagonism of muscarine-induced inhibition of spontaneously beating guinea pig right atria (Figure 1). In order to allow comparison of the results, the classical muscarinic receptor antagonist atropine was included in this study. The biological results were expressed as  $pA_2$  values determined from Schild plots.<sup>15</sup>

The most active member of the series, tetraamine 4, was selected for further pharmacological studies. Thus, nicotinic blocking activity was assessed by antagonism of carbachol-induced contractions of frog rectus abdominis (Figure 2). Furthermore, receptor specificity was studied also by investigating the responses elicited by 5-hydroxy-

tryptamine (5-HT) and histamine on guinea pig ileum and by norepinephrine on guinea pig left atria and rat vas deferens (results not shown).

## Results and Discussion

The results assembled in Table I indicate that all the compounds displayed significant activity toward muscarinic receptors of rat ileum and bladder and guinea pig atria. Figure 1 shows graphically that activity in atrial as well as in ileal and bladder preparations clearly depends on the carbon chain length separating the two inner nitrogens of the tetraamines. In fact, optimum activity in the atrium (force) is associated with an eight-carbon chain although compounds 3, 5, 6, and even 7 carrying seven, nine, 10, and 12 methylenes, respectively, retain high activity not significantly different from that of 4. This may indicate that the inner nitrogens of tetraamines interact with two receptor anionic sites separated by a distance of  $C_8$ , which are located at the boundary of hydrophobic area that can also accommodate longer carbon chains owing to their flexibility. On the other hand, compound 7, carrying 12 methylenes, displayed the highest activity in both ileum and bladder with an increase of almost 2 orders of magnitude compared to 1–6 (Table I). This activity was not significantly different from that in atria. The activity peaks in atria with eight-carbon chains and ileum and bladder with 12-carbon chains clearly indicate that tetraamines interact with muscarinic receptors by two distinct topographical mechanisms, which cannot be understood yet.<sup>16</sup> Whatever the interaction mechanism may be, present results clearly support the view that M-2 muscarinic receptors of peripheral organs are pharmacologically distinguishable owing to different structural requirements as revealed by the high selectivity toward cardiac muscarinic receptors displayed by tetraamines 1–6. An interesting observation is that the biological profile displayed in the ileum by 1–7 did not differ from that in bladder preparations.

Compound 4 showed a competitive mechanism of action in the concentration range investigated as revealed by the slope of the Schild plots and parallelism of the curves. It is clear that 4 is a potent antagonist at atrial muscarinic receptors, with a  $pA_2$  value of 8.13 not significantly different from that of atropine ( $pA_2$  8.69) and a high selectivity ratio (atria vs. ileum or bladder) of ca. 270 as shown in Table I. It suffices to say that gallamine, a claimed selective M-2 antagonist, is reported to have affinity for cardiac muscarinic receptors 10–15-fold higher than for ileal receptors.<sup>4,5</sup> Furthermore, the interpretation of results obtained with this neuromuscular blocking agent is complicated by the fact that its mechanism of action is not yet clear. In addition, the affinity of gallamine for cardiac M-2 receptors appears much lower than that of 4.<sup>4,5,8,17,18</sup>

As the most active and selective member of the series, 4 was selected to investigate receptor specificity. Thus, at a concentration of 10  $\mu$ M, compound 4 did not affect responses elicited by histamine and 5-hydroxytryptamine (5-HT) on guinea pig ileum. Similarly, at 10  $\mu$ M it failed to affect norepinephrine (NE) induced responses on guinea pig left atria whereas it gave a parallel shift to the right of the NE dose-response curve in isolated rat vas deferens

with a dose-ratio value of 2.71. This value corresponds to a  $-\log K$  value of 5.23, which clearly indicates weak antagonism at postsynaptic  $\alpha$ -adrenoreceptors. The biological profile of 4 at the nicotinic receptor was assessed on frog rectus abdominis, and the results are shown in Figure 2. It is clear that 4 inhibits nicotinic receptors with a noncompetitive mechanism because the maximum is depressed. Furthermore, this blockade was reversed after tissue washing. When the data in Figure 2 are plotted as a log dose-response curve, the  $IC_{50}$  value for 4, that is, the concentration required to inhibit 50% of the maximal response induced by carbachol, is 0.23  $\mu$ M.

In conclusion, compound 4 is the prototype of a novel class of selective inhibitors of M-2 muscarinic receptors in atrial preparations also displaying some degree of specificity. In fact, it does not affect 5-HT and histamine receptors whereas it causes a slight to moderate inhibition of  $\alpha$ -adrenergic and nicotinic receptors. This indicates that 4 may represent a valuable tool in the characterization of muscarinic receptor subtypes.<sup>16</sup>

## Experimental Section

**Chemistry.** Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian EM-390 instruments, respectively. Although the IR and NMR spectral data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were all consistent with the assigned structures. The microanalyses were performed by the Microanalytical Laboratory of the Department of Chemical Sciences of the University of Camerino, and the elemental compositions of the compounds agreed to within  $\pm 0.4\%$  of the calculated values.

***N,N'*-Bis[6-(benzyloxycarbonyl)amino]caproyl]-1, $\omega$ -alkanediamine (8–14).** The procedure adopted for the synthesis of 9 is described.

Ethyl chlorocarbonate (3.74 g, 35.0 mmol) was added dropwise to a stirred and cooled (5 °C) solution of *N*-(benzyloxycarbonyl)-6-aminocaproic acid (9.15 g, 34.49 mmol) and  $Et_3N$  (3.49 g, 34.49 mmol) in dioxane, followed after 30 min by the addition of a solution of 1,6-hexandiamine (2.0 g, 17.25 mmol) in dioxane (150 mL). After standing overnight, the mixture was poured into water (500 mL), and the white solid was filtered, washed with  $NaHCO_3$  saturated solution, 2 N HCl, and water, and then crystallized from MeOH to give the intermediate amide 9 ( $n = 6$ ) in 73% yield: mp 158–160 °C.

Similarly, 8 and 10–14 were obtained in 70–75% yields from suitable 1, $\omega$ -alkanediamine. Compounds 8–14 were used in the test step without further purification.

***N,N'*-Bis[6-(2-aminoethyl)-1, $\omega$ -alkanediamine (15–21).** These were prepared from 8–14 in 40–50% yields as follows. The appropriate amide (5.0 mmol) in MeOH (200 mL) was hydrogenated over 10% Pd on charcoal (0.5 g) for 60 h at room temperature and 30 psi of pressure. Following catalyst removal, the evaporation of the solvent gave a solid, which was dissolved in dry diglyme (200 mL) and treated with a 10 M solution of  $BH_3 \cdot MeSMe$  (1.0 mL) in dry diglyme (5 mL). After 15 h at 120 °C, excess borane was destroyed by careful addition of MeOH (5 mL), and the resulting mixture was left to stand for 5 h, treated with HCl gas for 30 min, and then heated at 120 °C for 4 h to give a white solid, which was filtered and recrystallized from MeOH to afford 15–21 as the tetrahydrochlorides which were used in the next step without further purification. Compound 16 ( $n = 6$ ) was identical in all respects (TLC, NMR, mp, IR) to a sample obtained by another method.<sup>11</sup>

***N,N'*-Bis[6-[(2-methoxybenzyl)amino]hexyl]-1, $\omega$ -alkanediamine Tetrahydrochlorides (1, 3–7).** These compounds were synthesized from 15, 17–21 (as free bases) and 2-methoxybenzaldehyde via the procedure described<sup>11</sup> for the carbon analogue (2) of benextramine.

The physical characteristics of 1–7 are reported in Table II. **Pharmacology. General Considerations.** Tissues from either rats (150–200 g) or guinea pigs (200–300 g) were suspended under 1 g tension in 10-mL organ baths containing physiological

(16) A detailed investigation of the carbon chain length ( $n > 12$ , Table I) separating the inner nitrogens of tetraamines will appear elsewhere together with the results of ongoing relevant research.

(17) Ellis, J.; Hoss, W. *Biochem. Pharmacol.* 1982, 31, 873.

(18) Choo, L. K.; Leung, E.; Mitchelson, F. *Can. J. Physiol. Pharmacol.* 1985, 63, 200

Table II

compd	mp, <sup>a</sup> °C	yield, %	recrystn solvent	formula <sup>b</sup>
1	224–226	61	MeOH	C <sub>33</sub> H <sub>60</sub> N <sub>4</sub> Cl <sub>4</sub> O <sub>2</sub> ·H <sub>2</sub> O
2	252–253 <sup>c</sup>	64	MeOH/ <i>i</i> -PrOH	C <sub>34</sub> H <sub>62</sub> N <sub>4</sub> Cl <sub>4</sub> O <sub>2</sub>
3	230–232	62	MeOH/ <i>i</i> -PrOH	C <sub>35</sub> H <sub>64</sub> N <sub>4</sub> Cl <sub>4</sub> O <sub>2</sub>
4	220	67	MeOH/ <i>i</i> -PrOH	C <sub>36</sub> H <sub>66</sub> N <sub>4</sub> Cl <sub>4</sub> O <sub>2</sub>
5	197–198	59	MeOH/ <i>i</i> -PrOH	C <sub>37</sub> H <sub>68</sub> N <sub>4</sub> Cl <sub>4</sub> O <sub>2</sub>
6	163–165	55	MeOH/ <i>i</i> -PrOH	C <sub>38</sub> H <sub>70</sub> N <sub>4</sub> Cl <sub>4</sub> O <sub>2</sub>
7	210	62	MeOH/ <i>i</i> -PrOH	C <sub>40</sub> H <sub>74</sub> N <sub>4</sub> Cl <sub>4</sub> O <sub>2</sub>

<sup>a</sup>The heating rate was 1 °C/min. <sup>b</sup>Analyses for C, H, N were within ±0.4% of the theoretical values required. <sup>c</sup>Ref 11 reported mp 242–243 °C.

salt solution (PSS) kept at 37 °C and aerated with 5% CO<sub>2</sub>–95% O<sub>2</sub>. The composition of PSS was as follows (mM): NaCl, 118; KCl, 4.7; MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.18; CaCl<sub>2</sub>, 2.52; KH<sub>2</sub>PO<sub>4</sub>, 1.18; NaHCO<sub>3</sub>, 23.8; dextrose, 11.7. Dose–response curves were constructed by the method of stepwise cumulative addition of the agonist. The concentration of agonist in the muscle chamber was increased approximately three-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Contractions were recorded by means of a force transducer connected to a two-channel Gemini polygraph.

**Rat Ileum, Bladder, and Vas Deferens.** All compounds listed in Table I were tested for muscarinic blocking activity in both ileum and bladder. A 20-mm-long portion of terminal ileum of male rats was taken at about 5 cm from the ileum–cecum junction. Alternatively, a 2-mm-wide longitudinal strip of bladder from the urethra to the apex of the bladder was cut, excluding the portion under the urethra orifice. Tissues were equilibrated for 30 min, and dose–response curves to furthrethonium iodide were obtained at 30-min intervals, the first one being discarded and the second one taken as a control. After a resting period of 15 min, tissues were incubated with the antagonist for 30 min and again exposed to the agonist. Responses were expressed as a percentage of the maximal response obtained in the control curve. Parallel experiments, in which tissues did not receive any antagonists, were run in order to correct for time-dependent changes in agonist sensitivity.<sup>19</sup> It was generally verified that the third dose–response curve was identical with the second because the change in dose ratio is less than 2, which usually represents a minimal correction.

The antagonist potency of compounds 1–7 and atropine at muscarinic receptors was expressed in terms of their pA<sub>2</sub> values according to Arunlakshana and Schild.<sup>15</sup> These values were calculated from the ratio of the doses (DR) of agonist causing 50% of the maximal response in the presence and in the absence of the test compound. The log (DR – 1) was calculated at three antagonist concentrations, and each concentration was tested at least five times.

Segments of ileum were used to determine the effects on the histamine- and 5-HT-induced responses by 4 at 10 μM concentration, following the procedure reported in ref 20. Dose–response

curves in the presence of 4 were not significantly different from control.

Tetraamine 4 was tested for postsynaptic α-adrenoreceptor blocking activity in the isolated rat vas deferens at a concentration of 10 μM, following the procedure reported in ref 21. The dose-ratio value in the presence of 4 measured at the level of 50% of the NE maximal response was 2.71.

**Guinea Pig Atria.** Muscarinic and postsynaptic α-adrenergic activities were studied on guinea pig right and left atria, respectively. When α-adrenergic antagonism was being studied, cocaine hydrochloride (10 μM) and propranolol hydrochloride (1 μM) were present in PSS to inhibit neuronal uptake and block β-adrenoreceptors, respectively. Tissues were equilibrated for 1 h, and cumulative dose–response curves to muscarine (right atria) and norepinephrine (left atria) were constructed. Following incubation with the antagonist for 30 min, a new dose–response curve to the agonist was obtained. Dose ratios were measured at the level of 50% inhibition of the force of left (preparations were driven at 1 Hz, 1-ms pulse duration, and 5-V stimulation) and right (tissues were allowed to beat spontaneously) atrial contractions.

The antagonist potency of 1–7 and atropine at cardiac muscarinic receptors was expressed in terms of their pA<sub>2</sub> values and calculated as reported for ileum and bladder preparations.

Tetraamine 4 at a concentration of 10 μM did not modify the NE-induced response.

**Frog Rectus Abdominis.** Compound 4 was tested for nicotinic blocking activity in isolated frog rectus abdominis via the procedure reported in ref 13. The results are shown in Figure 2.

**Statistical Evaluation.** Student's *t* test was used to assess the significance of the experimental results, and a level of *p* < 0.01 was taken as being statistically significant.

**Acknowledgment.** We thank Drs. A. Feriani, G. Garviraghi, and B. Valsecchi (Glaxo S.p.A., Verona) for valuable discussions and suggestions. This work was partially supported by a grant from the Ministry of Public Education.

**Registry No.** 1, 104807-44-5; 1 (free base), 104807-38-7; 2, 68536-02-7; 2 (free base), 102974-82-3; 3, 104807-45-6; 3 (free base), 104807-39-8; 4, 104807-46-7; 4 (free base), 104807-40-1; 5, 104807-47-8; 5 (free base), 104807-41-2; 6, 104807-48-9; 6 (free base), 104807-42-3; 7, 104807-49-0; 7 (free base), 104807-43-4; 8, 104807-16-1; 9, 23116-05-4; 10, 104807-17-2; 11, 104807-18-3; 12, 104807-19-4; 13, 104807-20-7; 14, 104807-21-8; 15, 104807-22-9; 15·4HCl, 104807-26-3; 15 (Schiff base), 104807-32-1; 16, 15518-45-3; 16·4HCl, 68536-00-5; 16 (Schiff base), 104807-33-2; 17, 104807-23-0; 17·4HCl, 104807-27-4; 17 (Schiff base), 104807-34-3; 18, 104807-24-1; 18·4HCl, 104807-28-5; 18 (Schiff base), 104807-35-4; 19, 104807-25-2; 19·4HCl, 104807-29-6; 19 (Schiff base), 104834-01-7; 20, 66907-35-5; 20·4HCl, 104807-30-9; 20 (Schiff base), 104807-36-5; 21, 104834-00-6; 21·4HCl, 104807-31-0; 21 (Schiff base), 104807-37-6; H<sub>2</sub>N(CH<sub>2</sub>)<sub>6</sub>NH<sub>2</sub>, 124-09-4; H<sub>2</sub>N(CH<sub>2</sub>)<sub>5</sub>NH<sub>2</sub>, 462-94-2; H<sub>2</sub>N(CH<sub>2</sub>)<sub>7</sub>NH<sub>2</sub>, 646-19-5; H<sub>2</sub>N(CH<sub>2</sub>)<sub>8</sub>NH<sub>2</sub>, 373-44-4; H<sub>2</sub>N(CH<sub>2</sub>)<sub>9</sub>NH<sub>2</sub>, 646-24-2; H<sub>2</sub>N(CH<sub>2</sub>)<sub>12</sub>NH<sub>2</sub>, 2783-17-7; ethyl chlorocarbonate, 541-41-3; 2-methoxybenzaldehyde, 135-02-4; *N*-(benzyloxy-carbonyl)-6-aminocaproic acid, 1947-00-8.

- (19) Furchgott, R. F. In *Catecholamines*; Blaschko, H., Muscholl, E., Eds.; Springer-Verlag: Berlin, 1972; p 283.  
 (20) Angeli, P.; Brasili, L.; Brancia, E.; Giardinà, D.; Guaglia, W.; Melchiorre, C. *J. Med. Chem.* 1985, 28, 1643.

- (21) Giardinà, D.; Angeli, P.; Brasili, L.; Gulini, U.; Melchiorre, C.; Strappaghetta, G. *Eur. J. Med. Chem.—Chim. Ther.* 1984, 19, 411.