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Anticholinergic Agents Based on Ariëns' Dual Receptor Site Theory: Nonester Antagonists

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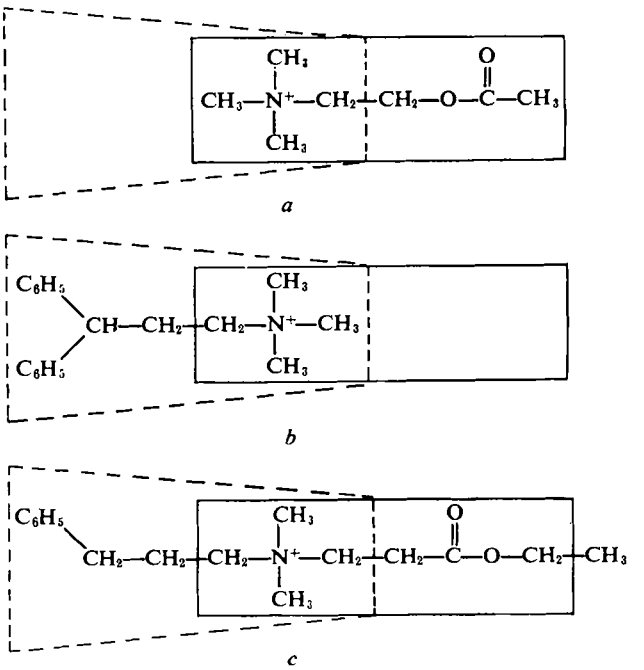
Abstract □ Additional compounds were prepared and evaluated for antimuscarinic activity as a test of Ariëns' dual receptor site theory. Consideration of the theory led to the conclusion that if an agonist moiety was added to the structure of classical muscarinic antagonists, then there might be an increased affinity for the receptor. Eleven such quaternary compounds, modeled after typical nonester antimuscarinic agents, were prepared. The pA_2 values against acetylcholine were determined on rat jejunum and compared to the activities of the classical quaternary antagonists. Only the compounds having an agonist moiety modeled after methylfurtrethonium consistently gave a significant increase in activity. The results do not allow a conclusion as to the validity of Ariëns' dual receptor site theory. Possible explanations for the results are considered.

Keyphrases □ Anticholinergic agents—synthesized, pharmacological screening, related to Ariëns' dual receptor site theory □ Receptor sites, Ariëns' dual theory—tested, nonester antagonists prepared, evaluated for antimuscarinic activity □ Antimuscarinic activity—nonester antagonists prepared and evaluated, Ariëns' dual receptor site theory tested

The long-term goal of the present research is to gain information concerning the nature of the subsites involved in binding various portions of agonist and antagonist molecules to the muscarinic receptor. Classical antimetabolite theory states that competitive antagonists should resemble agonists closely and bind with the same subsites. Many known pharmacodynamic antagonists do not closely resemble the agonist they antagonize, even though the inhibition is competitive and the antagonist is presumed to bind to the same receptor site. Furthermore, parallel modifications of the structure of agonists and antagonists often result in different effects on activity.

To account for these anomalies, Ariëns and Simonis (1–3) proposed that many known antagonists of muscarinic, histaminergic, and α-adrenergic agents are binding to sets of subsites on the receptor surface which overlap but do not include all of the subsites used by the agonist. If acetylcholine is bound to a portion of the muscarinic receptor surface as represented in Scheme I*a*, then Ariëns proposed that a quaternary antagonist might bind as shown in Scheme I*b*.

If the classical antimuscarinic agents are bound to the receptor as shown in Scheme I*b*, then the esteratic subsite normally involved with acetylcholine is left exposed. Compounds that mimic the structure of classical antagonists and have an additional agonist moiety connected through the quaternary nitrogen might have greater affinity if all portions could bind simultaneously to their respective subsites. Ariëns (3) reported that ethyl 3-[methyl(phenylpropyl)amino]-propionate methobromide was a weak antagonist, presumably binding to the receptor as shown in Scheme I*c*



Scheme I—Proposed modes of binding of agonists and antagonists to the muscarinic receptor

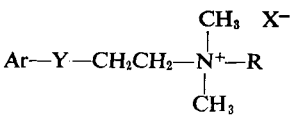


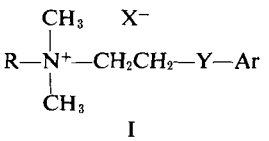
Table I—Physical Properties and Elemental Analyses of New Compounds

Compound Number	Ar—Y	R	X^-	Melting Point[a]	Recrystallization Solvent[b]	Empirical Formula	—Analysis, %— Calc.	Found
1	$PhCH_2$—	Acetoxyethyl	I	97.5–99°	D–E	$C_{15}H_{24}INO_2$	C 47.75	47.59
							H 6.41	6.46
							N 3.71	3.78
2	$PhCH_2$—	Methylfurfuryl	Br	102–104°	A–T	$C_{17}H_{24}BrNO$	C 60.36	60.44
							H 7.15	7.18
							N 4.14	4.09
3	Ph_2CH—	Acetoxyethyl	Br	136.5–138°	D–E	$C_{21}H_{28}BrNO_2$	C 62.07	61.83
							H 6.95	6.98
							N 3.45	3.50
4	Ph_2CH—	Methylfurfuryl	Br	170–172°	B–C	$C_{23}H_{28}BrNO$	C 66.65	66.40
							H 6.81	6.99
							N 3.38	3.11
5	Ph_2CHO—	Butyl	Br	125–126°	B–A	$C_{21}H_{30}BrNO$	C 64.28	64.52
							H 7.71	7.74
							N 3.57	3.55
6	Ph_2CHO—	Acetoxyethyl	Br	105–107°	A–M	$C_{21}H_{28}BrNO_3$	C 59.71	59.51
							H 6.68	6.62
							N 3.31	3.53
7	Ph_2CHO—	Carbamyloxyethyl	Cl	148–150°	B	$C_{20}H_{27}ClN_2O_3$	C 63.39	63.28
							H 7.18	7.13
							N 7.39	7.28
8	Ph_2CHO—	Methylfurfuryl	Br	93–95°	B–C	$C_{23}H_{28}BrNO_2$	C 64.19	64.03
							H 6.56	6.53
							N 3.26	3.19
9	$Ph_2C(OH)$—	Acetoxyethyl	Br	181–183°	D–E	$C_{21}H_{28}BrNO_3$	C 59.71	59.34
							H 6.68	6.87
							N 3.31	3.09
10	$Ph_2C(OH)$—	Carbamyloxyethyl	Cl	217–219°	A–E	$C_{20}H_{27}ClN_2O_3$	C 63.39	63.10
							H 7.18	7.30
							N 7.39	7.60
11	$Ph_2C(CONH_2)$—	Butyl	Br	233–235°	I–E	$C_{22}H_{31}BrN_2O$	C 63.00	62.77
							H 7.45	7.63
							N 6.68	6.58
12	$Ph_2C(CONH_2)$—	Acetoxyethyl	Br	210–212°	I–E	$C_{22}H_{29}BrN_2O_3$	C 58.80	58.61
							H 6.50	6.71
							N 6.23	6.11
13	$Ph_2C(CONH_2)$—	Carbamyloxyethyl	Cl	202–204°	B–C	$C_{21}H_{28}ClN_3O_3$	C 62.13	61.91
							H 6.95	6.93
							N 10.35	10.17

[a] Melting points were determined on a Thomas-Hoover melting-point apparatus and are uncorrected. [b] A = ethyl acetate, B = benzene, C = chloroform, D = diethyl ether, E = ethanol, I = 2-propanol, M = methyl ethyl ketone, and T = tetrahydrofuran. Microanalyses were performed by Microtek, Inc., Skokie, Ill.

(3); however, this compound was not compared to the corresponding antagonist lacking the agonist moiety. Previous work in this laboratory showed that compounds modeled after the ester-type antagonist lachesine and which contained an agonist moiety did not exhibit enhanced affinity (4, 5).

In the present work, 19 compounds containing both an agonist and a nonester antagonist moiety were prepared. These have the general structure I. The R group was acetoxyethyl, carbamyloxyethyl, or 5-methylfurfuryl to duplicate the common potent agonists acetylcholine, carbachol, or methylfurtrethonium, re-



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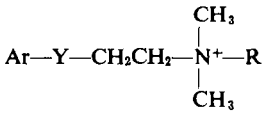
spectively. In addition, compounds having R equal to methyl or butyl were prepared as standards for comparing the effect of the specific agonist moieties. The Ar—Y group was $PhCH_2$—, Ph_2CH—, $Ph_2C(OH)$—, Ph_2CHO—, or $Ph_2C(CONH_2)$— to provide the aralkyl groups found in typical nonester antagonists (Ph = phenyl).

EXPERIMENTAL

Syntheses—The compounds were prepared by alkylation of the tertiary amine containing either the agonist or antagonist portion with the appropriate halide (4). The presence of strong N^+—H absorption bands in the IR spectra of the crude reaction products indicated that dehydrohalogenation was always an important side reaction. In some cases the tertiary amine hydrohalides were isolated and identified as the major products. A variety of reaction conditions were used. In certain instances, reaction of the amine and halide without solvent for 1–2 weeks at room temperature proved to be the most satisfactory method. Others required reflux conditions in a suitable solvent. In all cases, purification was difficult, and many trials gave oils which could not be induced to crystallize. The structures assigned to the products were confirmed by IR spectra and elemental analyses. The physical properties and analytical data are presented in Table I.

It was necessary to use one variation for the synthesis of Compound 2 (Table I). Treatment of *N*,*N*,5-trimethylfurfurylamine with 3-phenylpropyl bromide gave a quaternary bromide salt which resisted all attempts at crystallization. *N*,5-Dimethylfurfurylamine was reacted with 3-phenylpropyl bromide. The tertiary amine product was isolated and treated with methyl iodide to give the quaternary iodide salt of the desired structure. The intractable oil was "false seeded" with the iodide salt, and crystallization was in-

Table II—Antimuscarinic Activity of the Compounds on Rat Jejunum



Compound	Ar—Y	R	$pA_2 \pm SE$[a]
1	$PhCH_2$—	Methyl	4.92 ± 0.11 (20)
2	$PhCH_2$—	Acetoxyethyl	4.85 ± 0.10 (19)
3	$PhCH_2$—	Methylfurfuryl	6.15 ± 0.15 (8)
4	Ph_2CH—	Methyl	6.49 ± 0.14 (20)
5	Ph_2CH—	Acetoxyethyl	7.06 ± 0.13 (18)
6	Ph_2CH—	Methylfurfuryl	7.26 ± 0.22 (12)
7	Ph_2CHO—	Methyl	7.40 ± 0.18 (16)
8	Ph_2CHO—	Butyl	7.45 ± 0.11 (10)
9	Ph_2CHO—	Acetoxyethyl	7.32 ± 0.10 (12)
10	Ph_2CHO—	Carbamyloxyethyl	7.47 ± 0.16 (12)
11	Ph_2CHO—	Methylfurfuryl	7.90 ± 0.12 (12)
12	$Ph_2C(OH)$—	Methyl	6.85 ± 0.07 (24)
13	$Ph_2C(OH)$—	Butyl	6.77 ± 0.17 (12)
14	$Ph_2C(OH)$—	Acetoxyethyl	6.95 ± 0.14 (12)
15	$Ph_2C(OH)$—	Carbamyloxyethyl	6.79 ± 0.49 (6)
16	$Ph_2C(CONH_2)$—	Methyl	8.22 ± 0.10 (14)
17	$Ph_2C(CONH_2)$—	Butyl	8.29 ± 0.11 (10)
18	$Ph_2C(CONH_2)$—	Acetoxyethyl	8.50 ± 0.15 (10)
19	$Ph_2C(CONH_2)$—	Carbamyloxyethyl	8.41 ± 0.19 (6)

[a] Numbers in parentheses refer to the number of animals used in each determination.

duced. The solid quaternary bromide was used for pharmacological testing.

Pharmacology—To evaluate the affinity of the compounds for the muscarinic receptor, pA_2 values were measured by standard techniques. Small strips of muscle from the rat jejunum were placed in an isolated organ bath containing oxygenated Tyrode's solution, and one end was attached to a force transducer. Four muscle strips were run simultaneously, and the contractions were recorded on a four-channel polygraph[1]. Cumulative dose–response curves to acetylcholine were obtained on each strip. This curve was then repeated in the presence of three or four different concentrations of the compound being evaluated. From 6 to 24 different animals were used with each compound. Linear regression analysis of the data obtained yielded the pA_2 values of the compounds (Table II).

RESULTS AND DISCUSSION

Examination of Table II shows that the 5-methylfurfuryl group in Compounds 3, 6, and 11 contributed significantly to the affinity of the compounds compared to the corresponding antagonist having only methyl groups on the cationic head (Compounds 1, 4, and 7, respectively). However, the acetoxyethyl and carbamyloxyethyl groups failed to yield a consistent increase in affinity compared to the model compounds. The acetoxyethyl group gave a significant increase in affinity only in the case of Compound 5. None of the carbamyloxyethyl compounds had significantly increased affinity. The change of methyl to butyl on the cationic head had little effect on affinity. The butyl group would not be expected to give a highly specific binding to the esteratic site of the muscarinic receptor.

The results do not allow a distinct conclusion that Ariëns' hypothesis of the dual receptor sites for agonists and antagonists is either correct or incorrect. It was pointed out in earlier work regarding the ester-type antagonists that the angle between the regions used by the agonist and classical antagonists may be too sharp to allow occupation of the esteratic subsite, anionic subsite, and the aromatic ring-binding subsites simultaneously (5). Thus, the agonist portion would be in an unfavorable position in relation to the esteratic subsite unless strained conformations were induced in the molecule.

Other alternative explanations which must be considered include:

1. Interactions between the agonist and antagonist ends of the molecule might result in conformational changes not found in the separate agonist and antagonist molecules.
2. The entropy effects might counterbalance the favorable enthalpy effect of utilizing an additional binding site in the case of the acetoxy- or carbamyloxyethyl groups.
3. The nonester antagonists may be blocking contractions at a point beyond the muscarinic receptor. Two potential sites for blocking the action of norepinephrine were proposed by Wohl *et al.* (6) and Moran *et al.* (7) in the case of the α-adrenergic receptor.

Further work is needed to distinguish between these possibilities.

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