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Binding and functional properties of antimuscarinics of the hexocyclium/sila-hexocyclium and hexahydro-diphenidol/hexahydro-sila-diphenidol type to muscarinic receptor subtypes

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1 In an attempt to assess the structural requirements for the muscarinic receptor selectivity of hexahydro-diphenidol (hexahydro-difenidol) and hexahydro-sila-diphenidol (hexahydro-sila-difenidol), a series of structurally related C/Si pairs were investigated, along with atropine, pirenzepine and methoctramine, for their binding affinities in NB-OK 1 cells as well as in rat heart and pancreas.

2 The action of these antagonists at muscarinic receptors mediating negative inotropic responses in guinea-pig atria and ileal contractions has also been assessed.

3 Antagonist binding data indicated that NB-OK 1 cells (M_1 type) as well as rat heart (cardiac type) and pancreas (glandular/smooth muscle type) possess different muscarinic receptor subtypes.

4 A highly significant correlation was found between the binding affinities of the antagonists to muscarinic receptors in rat heart and pancreas, respectively, and the affinities to muscarinic receptors in guinea-pig atria and ileum. This implies that the muscarinic binding sites in rat heart and the receptors in guinea-pig atria are essentially similar, but different from those in pancreas and ileum.

5 The antimuscarinic potency of hexahydro-diphenidol and hexahydro-sila-diphenidol at the three subtypes was influenced differently by structural modifications (e.g. quaternization). Different selectivity profiles for the antagonists were obtained, which makes these compounds useful tools to investigate further muscarinic receptor heterogeneity. Indeed, the tertiary analogues hexahydro-diphenidol (HHD) and hexahydro-sila-diphenidol (HHSiD) had an M_1 = glandular/smooth muscle > cardiac selectivity profile, whereas the quaternary analogues HHD methiodide and HHSiD methiodide were M_1 preferring (M_1 > glandular/smooth muscle, cardiac).

Introduction

Three muscarinic receptors with different selectivities for antagonists have been described in binding studies as well as in *in vitro* and *in vivo* pharmacological assays. M_1 receptors are above all found in the nervous system and are characterized by a high affinity for pirenzepine (Hammer *et al.*, 1980; Birdsall *et al.*, 1980; Brown *et al.*, 1980; Hammer & Giachetti, 1982; Lambrecht *et al.*, 1988b). M_2 receptors, which show low affinity for pirenzepine, have been further subdivided into cardiac type and glandular/smooth muscle subtypes by use of selective antagonists such as 4-diphenylacetoxy-*N*-methyl-piperidine methiodide (4-DAMP) (Barlow *et al.*, 1976; Waelbroeck *et al.*, 1987a; Doods *et al.*, 1987), hexahydro-diphenidol (hexahydro-difenidol) and hexahydro-sila-diphenidol (hexahydro-sila-difenidol) (Mutschler & Lambrecht, 1984; Waelbroeck *et al.*, 1987b; Lambrecht *et al.*, 1988a, c), AF-DX 116 (Hammer *et al.*, 1986; Giachetti *et al.*, 1986; Doods *et al.*, 1987) and methoctramine (Melchiorre *et al.*, 1987; Michel & Whiting, 1988).

Among these selective muscarinic antagonists, hexahydro-diphenidol (*3a*) and hexahydro-sila-diphenidol (*3b*) have been shown to have high affinity

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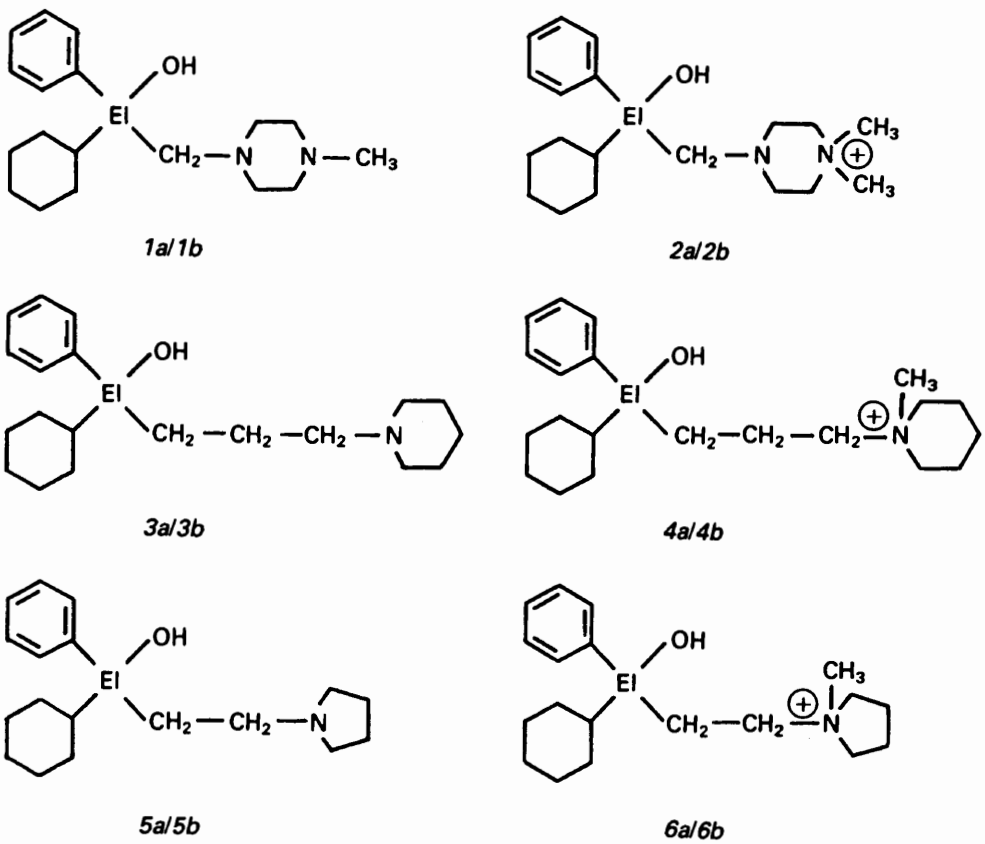

a: El = C, b: b: El = Si

Figure 1 Structural formula of antagonists studied: demethyl-hexocyclium (*1a*), demethyl-sila-hexocyclium (*1b*), hexocyclium (*2a*), sila-hexocyclium (*2b*), hexahydro-diphenidol (*3a*), hexahydro-sila-diphenidol (*3b*), *N*-methyl-hexahydro-diphenidol (*4a*), *N*-methyl-hexahydro-sila-diphenidol (*4b*), procyclidine (*5a*), sila-procyclidine (*5b*), tricyclamol (*6a*), and sila-tricyclamol (*6b*).

for M_1 receptors in neuronal tissues as well as for glandular/smooth muscle receptors in exocrine glands and smooth muscle, but a much lower affinity for cardiac receptors (Mutschler & Lambrecht, 1984; Waelbroeck *et al.*, 1987b; Lambrecht *et al.*, 1988a,c). In order to assess the structural requirements for the observed selectivity of *3a/3b*, the binding characteristics of a series of C/Si pairs structurally related to *3a/3b* were determined (compounds *1a/1b*, *2a/2b*, *4a/4b–6a/6b*; Figure 1). These experiments were carried out using a NB-OK 1 cell line (M_1 receptors type) and rat heart (cardiac receptors type) and pancreas (glandular/smooth muscle receptors type) homogenates (Waelbroeck *et al.*, 1987a,b,c). Furthermore the binding affinities of compounds *1a/1b–6a/6b* to glandular/smooth muscle and cardiac receptor types were compared with the functional antimuscarinic properties in guinea-pig isolated ileum and atria. Atropine, pirenzepine and methoctramine were used as reference drugs.

The experiments were carried out using the racemates of compounds *1a/1b* to *6a/6b* but some data of functional experiments with the individual enantiomers of *5a/5b* and *6a/6b* have been published elsewhere (Tacke *et al.*, 1986; 1987a) and binding studies are in progress.

Methods

Radioligand binding studies

Tissue preparations Male Wistar rats (200–250 g) were killed by decapitation and the pancreas and heart were immediately removed.

The pancreas was minced with scissors and homogenized in 8 ml per pancreas of 300 mM sucrose enriched with 0.2 mg ml^{-1} bacitracin and 500 kallikrein inhibitor units ml^{-1} of aprotinin, with a glass-

Teflon homogenizer (7 up and down strokes). The resulting homogenate was filtered on two layers of medical gauze, and immediately diluted 11 fold with the incubation buffer (66 mM sodium phosphate buffer (pH 7.4) enriched with 2.6 mM $MgCl_2$ and with 1.32% bovine serum albumin, 0.24 mg ml^{-1} bacitracin and 600 kallikrein inhibitor units ml^{-1} aprotinin).

The hearts were rinsed in isotonic NaCl, then homogenized in 2.5 ml of Tris-HCl buffer (pH 7.5) enriched with 250 mM sucrose, in an Ultraturrax homogenizer (5 s at maximum setting). The resulting homogenate was diluted 8 fold with the same buffer and filtered on two layers of medical gauze.

NB-OK 1 cells were cultured in RPMI 1640 medium, enriched with 10% foetal calf serum, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin, as described previously (Waelbroeck *et al.*, 1988). For [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) binding experiments, the cells were rinsed, detached and centrifuged in 20 mM sodium phosphate buffer (pH 7.4) containing 156 mM NaCl and 1 mM EDTA, resuspended and homogenized in 20 mM Tris-HCl buffer (pH 7.5) enriched with 5 mM $MgCl_2$ and stored in liquid nitrogen.

Binding experiments: On rat pancreas homogenates, 1 ml of diluted homogenate (800 to 1000 μg protein per assay) was added to 200 μl of [^{3}H]-NMS and unlabelled drugs (in water) to obtain final concentrations of 50 mM sodium phosphate buffer (pH 7.4), 2 mM $MgCl_2$, 1% bovine serum albumin, 0.2 mg ml^{-1} bacitracin and 500 kallikrein inhibitor units ml^{-1} of aprotinin. The [^{3}H]-NMS concentration was 240 pM (with a K_D value of 120 pM in pancreas). This incubation was prolonged for 4 h at 25°C to allow equilibration of tracer binding to pancreas receptors (Waelbroeck *et al.*, 1987b).

On rat cardiac homogenates, 80 μl of homogenate (400 to 500 μg of protein) was used per assay, in a total volume of 1.2 ml containing (final concentrations): 50 mM sodium phosphate buffer (pH 7.4), 2 mM $MgCl_2$, 1% bovine serum albumin, and the indicated concentrations of [^{3}H]-NMS, and unlabelled drugs. The [^{3}H]-NMS concentration was 1.0 nM (with a K_D value of 0.5 nM in heart). The incubation period was 2 h at 25°C, sufficient to allow equilibrium binding of [^{3}H]-NMS in this tissue (Waelbroeck *et al.*, 1987a,b).

On human NB-OK 1 homogenates, 80 μl of homogenate (80 μg of protein) was used per assay, in a total volume of 1.2 ml containing (final concentrations): 50 mM sodium phosphate buffer (pH 7.4), 2 mM $MgCl_2$, 1% bovine serum albumin, and the indicated concentrations of [^{3}H]-NMS, and unlabelled drugs. The [^{3}H]-NMS concentration was 240 pM (with a K_D value of 120 pM in NB-OK 1 homogenates). An incubation period of 2 h at 25°C was sufficient to allow equilibrium binding of [^{3}H]-NMS in this tissue (Waelbroeck *et al.*, 1988).

Non-specific [^{3}H]-NMS binding, using pancreas, heart and NB-OK 1 homogenates was defined as [^{3}H]-NMS binding in the presence of 1 μM atropine. In our binding conditions, non-specific binding represented less than 5% of total tracer binding, and was fully accounted for by binding to GF/C glass-fibre filters (see below).

To separate bound and free [^{3}H]-NMS, in pancreas, heart or NB-OK 1 homogenates, 2 ml of ice-cold 50 mM sodium phosphate buffer (pH 7.4) was added to each sample, followed by immediate filtration on GF/C glass-fibre filters (Whatman, Maidstone, Kent, England) presoaked overnight in 0.05% polyethyleneimine. The filters were rinsed three times with the same phosphate buffer, dried, and the radioactivity counted by liquid scintillation.

Analysis of binding data All competition curves were repeated at least three times, in duplicate. IC_{50} values were determined by a computer-aided procedure described by Richardson & Humrich (1984), assuming the existence of only one receptor subtype. Experimental data points were within 3% of the expected values, assuming that the molecules investigated competed with [^{3}H]-NMS for binding to a single site.

K_i values were determined from IC_{50} values, by the Cheng & Prusoff (1973) equation which assumes competitive inhibition of tracer binding to a single receptor subtype. The [^{3}H]-NMS K_D value for the three systems investigated was determined in separate experiments, as described by Waelbroeck *et al.* (1987a,b; 1988). The pK_i values, indicated in Table 1, corresponded to $-\log K_i$ values.

The standard deviation of pIC_{50} ($-\log IC_{50}$) determination was equal to or below 0.10 log unit. Repeated determinations of [^{3}H]-NMS K_D values were within 10% of each other. This error should be added to errors in IC_{50} determinations, since [^{3}H]-NMS K_D values were used to calculate pK_i values. We therefore estimated the standard deviation of pK_i values as being of approximately 0.15 log unit (40% of K_i value).

Pharmacological studies

General considerations Guinea-pigs (300–450 g) of either sex were killed by cervical dislocation and the organs required were set up under 0.5 g tension in 6 ml organ baths containing physiological salt solution (PSS) and gassed with 5% CO_2/95% O_2. The composition of PSS was as follows (mM): NaCl 137.0, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, glucose 5.6. All experiments

were conducted at pH 7.4, 32°C. Arecaidine propargyl ester (Mutschler & Lambrecht, 1984; Barlow & Weston-Smith, 1985), except where indicated, was used as the agonist.

After 1 h equilibration, concentration-response curves were obtained by cumulative addition of the agonists (van Rossum, 1963). When these responses were regular the tissues were exposed to a solution of antagonist and each concentration of antagonist was allowed to equilibrate before repeating the concentration-response curve.

Guinea-pig isolated ileum Strips of ileal longitudinal muscle (1.5 cm) were prepared according to Paton & Zar (1968). Tissue responses were measured as isotonic contractions with an electromechanical transducer connected to a Hellige amplifier and a Rikadenki recorder. The responses were then calculated as a percentage of the maximum response obtained to arecaidine propargyl ester and plotted against the logarithm of the agonist concentration, and EC_{50} values were determined for the control and the antagonist shifted concentration-response curves.

Guinea-pig isolated atria Left atria were electrically paced, by use of platinum electrodes (2 Hz, 3 ms duration, supramaximal voltage). Atrial responses to the agonists were measured as changes in isometric tension, and these effects were expressed as the percentage inhibition of the force of contraction. EC_{50} values were determined as described for the ileum.

Antagonist affinities (pA_2) Concentration-response curves were repeated in the presence of at least 3 concentrations (log interval = 0.48) of antagonists, allowing 15–45 min equilibration time. Each concentration of antagonist was tested 3 to 5 times and dose-ratios at EC_{50} values of agonists were calculated. These dose-ratios were pooled for each concentration of antagonist and the slope of the Arunlakshana and Schild plots were determined by linear regression using the method of least squares. pA_2 values were estimated by fitting to the data the best straight line with a slope of unity (Arunlakshana & Schild, 1959; Tallarida *et al.*, 1979).

Statistical analysis The data are presented as means ± s.e.mean of n experiments. Differences between mean values were tested for statistical significance by means of Student's t test, accepting $P < 0.05$ as being significant.

Compounds

[^{3}H]-N-methylscopolamine ([^{3}H]-NMS, 72 Ci mmol^{-1}) was obtained from Amersham International (Bucks, England). Atropine, carbachol, bacitracin and polyethylenimine were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). Aprotinin (Trasylol) was obtained from Bayer, Belgium. Pirenzepine was a gift of Thomae (F.R.G.) and procyclidine (*5a*) was kindly provided by Deutsche Wellcome (F.R.G.). Methoctramine was kindly donated by Dr C. Melchiorre, University of Bologna, Italy. Arecaidine propargyl ester was synthesized in one of our laboratories (Mutschler & Hultzsch, 1973).

Demethyl-hexocyclium [1-cyclohexyl-1-phenyl-2-(1-methylpiperazin-4-yl)-1-ethanol, *1a*], demethyl-sila-hexocyclium [cyclohexyl((1-methylpiperazin-4-yl)methyl)phenylsilanol, *1b*], hexocyclium methyl sulphate [4-(2-cyclohexyl-2-hydroxy-2-phenylethyl)-1,1-dimethylpiperazinium methyl sulphate, *2a* $CH_3OSO_3^-$], sila-hexocyclium methyl sulphate [4-((cyclohexylhydroxyphenylsilyl)methyl)-1,1-dimethylpiperazinium methyl sulphate, *2b* $CH_3OSO_3^-$], hexahydro-diphenidol hydrochloride [1-cyclohexyl-1-phenyl-4-piperidino-1-butanol hydrochloride, *3a* · HCl], hexahydro-sila-diphenidol hydrochloride [cyclohexylphenyl(3-piperidinopropyl)silanol hydrochloride, *3b* · HCl], sila-procyclidine [cyclohexylphenyl(2-pyrrolidinoethyl)silanol, *5b*], and tricyclamol chloride [1-(3-cyclohexyl-3-hydroxy-3-phenylpropyl)-1-methylpyrrolidinium chloride, *6a* Cl^-] were prepared according to the literature: *1a* and *2a* $CH_3OSO_3^-$ (Zaugg *et al.*, 1958); *1b* and *2b* $CH_3OSO_3^-$ (Tacke *et al.*, 1989); *3a* · HCl and *3b* · HCl (Tacke *et al.*, 1985); *5b* (Tacke *et al.*, 1983; Tacke *et al.*, 1987a); *6a* Cl^- (Murfitt, 1956).

Synthetic chemistry *N*-methyl-hexahydro-diphenidol iodide [1-(4-cyclohexyl-4-hydroxy-4-phenylbutyl)-1-methylpiperidinium iodide, *4a* I^-], *N*-methyl-hexahydro-sila-diphenidol iodide [1-(3-(cyclohexylhydroxyphenylsilyl)propyl)-1-methylpiperidinium iodide, *4b* I^-], and sila-tricyclamol iodide [1-(2-((cyclohexylhydroxyphenyl)silyl)ethyl)-1-methylpyrrolidinium iodide, *6b* I^-] were prepared as follows: freshly distilled methyl iodide (18 mmol) was added under an atmosphere of dried nitrogen to a solution of *3a*, *3b* or *5b* (8 mmol) in dry ethanol (50 ml). After the reaction mixture was stirred for 3 h at 30°C, dry *n*-pentane (75 ml (*3a*, *3b*) and 150 ml (*5b*), respectively) was added, and the mixture was stirred for 1.5 h at 20°C. Thereafter, the precipitate was collected by filtration and then recrystallized from ethanol/*n*-pentane. (After dissolving the precipitate in dry ethanol, the fourfold (*3a*, *3b*) and twofold (*5b*) volume, respectively, of dry *n*-pentane was added dropwise to the solution). After drying the crystals *in vacuo* (because of their hygroscopic nature, the products *4b* and *6b* needed an especially

Table 1 Antagonist affinities (pK_i values) obtained in [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) binding studies in NB-OK 1 (M_1 receptors), pancreas (glandular/smooth muscle receptors) and cardiac (cardiac receptors) homogenates

Antagonist	*NB-OK 1 cells*	*Pancreas*	*Heart*
1a Demethyl-hexocyclium	8.0	7.4	6.7
1b Demethyl-sila-hexocyclium	7.9	7.4	6.6
2a Hexocyclium	8.8	8.4	7.7
2b Sila-hexocyclium	8.9	8.4	7.6
3a Hexahydro-diphenidol	7.9	7.9	6.8
3b Hexahydro-sila-diphenidol	7.9	7.8	6.8
4a HHD methiodide	8.5	7.9	8.1
4b HHSiD methiodide	8.8	8.2	8.0
5a Procyclidine	8.1	7.6	7.1
5b Sila-procyclidine	8.5	7.9	7.4
6a Tricyclamol	8.9	8.0	7.8
6b Sila-tricyclamol	9.1	8.2	8.0
Atropine	9.5	9.4	9.0
Pirenzepine	8.2	6.8	6.5
Methoctramine	7.3	5.9	8.2

The numbers show the mean estimate of the value of $-\log$ inhibitor constant (pK_i) for $n = 3$ experiments. The standard deviation was approximately 0.15 log unit (see Methods).
HH(Si)D methiodide = N-methyl-hexahydro-(sila-) diphenidol iodide.

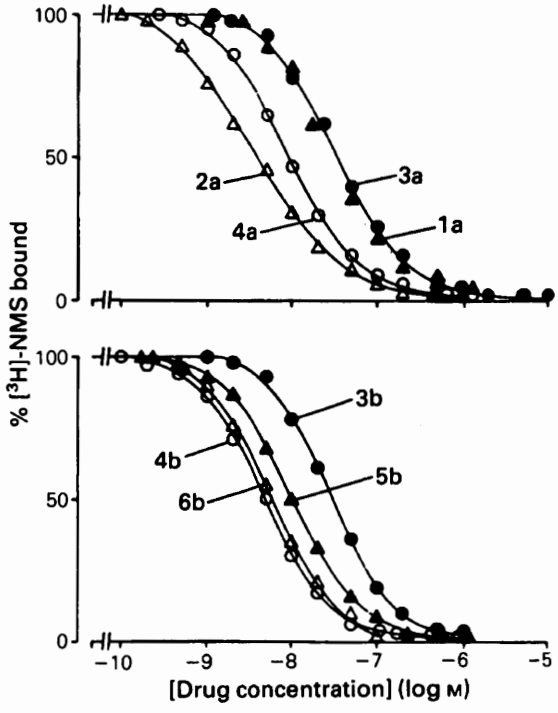

Figure 2 [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) competition curves in human neuroblastoma NB-OK 1 cells. [^{3}H]-NMS binding to NB-OK 1 cells homogenates was measured in the absence or presence of 1a (▲), 2a (△), 3a (●), 4a (○) (top panel) or of the corresponding sila-analogues 3b (●), 4b (○), 5b (▲) or 6b (△) (bottom panel), as indicated in Methods. Average of 3 to 4 experiments performed in duplicate.

careful drying) analytically pure products were obtained [characterized by ^{1}H n.m.r., ^{13}C n.m.r. and FAB MS measurements (data not given) as well as by elemental analyses].

4a: $C_{22}H_{36}INO$ (457.4), yield 89%, m.p. 194°C; found: C, 57.8; H, 8.1; N, 3.1; calculated: C, 57.77; H, 7.93; N, 3.06%.

4b: $C_{21}H_{36}INOSi$ (473.5), yield 65%, m.p. 119–121°C; found: C, 53.3; H, 7.7; N, 3.1; calculated: C, 53.27; H, 7.66; N, 2.96%.

6b: $C_{19}H_{32}INOSi$ (445.5), yield 92%, m.p. 128–129°C; found: C, 51.6; H, 7.3; N, 3.1; calculated: C, 51.23; H, 7.24; N, 3.14%.

All antagonists studied were racemates.

Results

Radioligand binding studies in NB-OK 1, heart and pancreas homogenates

All compounds antagonized [^{3}H]-NMS binding to muscarinic receptors. Competition curves with the C/Si pairs *1a/1b–6a/6b* did not deviate significantly from results expected for competitive inhibition of tracer binding to a single receptor. Representative competition curves are shown in Figures 2 to 4 and pK_i values are given in Table 1.

Atropine, pirenzepine and the quaternary compounds *4a/4b* and *6a/6b* exhibited similar affinities for muscarinic receptors in pancreas and heart. At both receptors the following rank order of potencies was obtained: atropine > *4a/4b* = *6a/6b* > pirenzepine.

The affinities obtained for compounds *1a/1b*, *2a/2b*, *3a/3b* and *5a/5b* were significantly greater for receptors in pancreas as compared to receptors in heart, and the following rank orders of potencies were obtained; heart: *2a/2b* > *5a/5b* > *3a/3b* ≥ *1a/1b*; pancreas: *2a/2b* > *3a/3b* ≥ *5a/5b* ≥ *1a/1b*. In contrast, the pK_i value obtained for methoctramine was markedly lower in the pancreas as compared to heart.

Pirenzepine, compounds *1a/1b*, *2a/2b*, *4a/4b*, *5a/5b*, and *6a/6b* recognized preferentially the receptors expressed by NB-OK 1 cells, as compared to

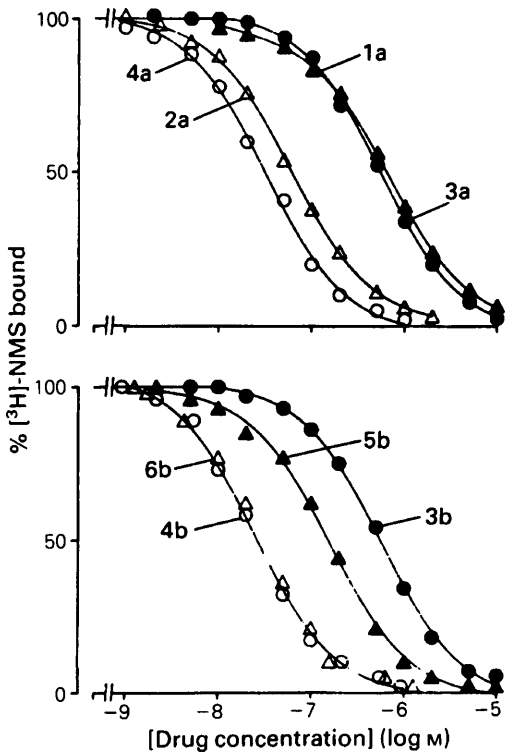

Figure 3 [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) competition curves in rat heart. Details as in Figure 1, but with heart homogenates.

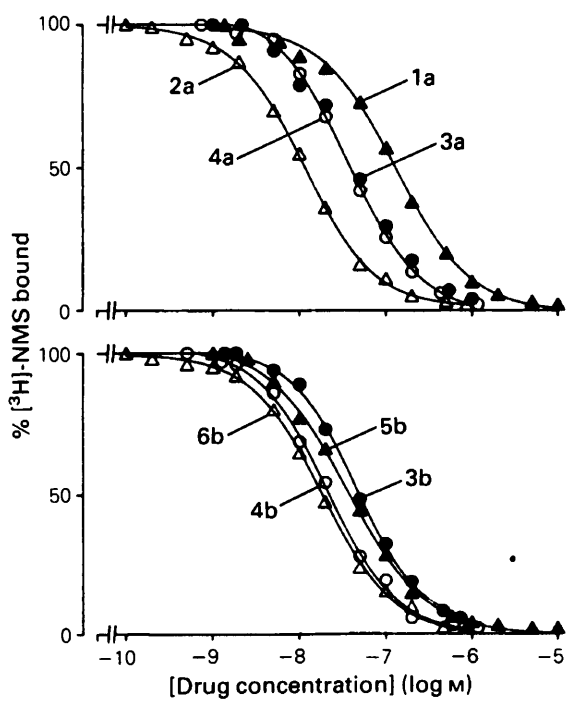

Figure 4 [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) competition curves in rat pancreas. Details as in Figure 1, but with pancreas homogenates.

heart and pancreas muscarinic receptors. The following rank order of potencies was obtained: *6a/6b, 2a/2b, 4a/4b* ≥ pirenzepine = *5a/5b* ≥ *1a/1b*. The following rank order of M_1 selectivities (pK_i in NB-OK 1 cells vs pK_i in pancreas) was obtained: pirenzepine > *6a/6b* ≥ *4a/4b* ≥ *1a/1b* = *5a/5b* ≥ *2a/2b*.

Methoctramine and *3a/3b* presented a different selectivity pattern. Whereas methoctramine recognized the NB-OK 1 receptors with an affinity intermediate between its affinity for cardiac and pancreas receptors, compounds *3a/3b* exhibited the same high affinity for NB-OK 1 and pancreas receptors and a much lower affinity for the receptors in the heart.

Isolated tissue experiments on atria and ileum from guinea-pig

All compounds antagonized the negative inotropic effects in electrically stimulated atria and the contractions of the ileum induced by arecaidine propargyl ester or carbachol. There was a concentration-dependent parallel shift to the right of agonist concentration-response curves without either basal tension or maximum response being affected. In all experiments Schild plots were linear through the concentration range tested for each antagonist, and slopes were not significantly different from unity ($P > 0.05$). Thus, all compounds were apparently simple competitive antagonists in both atrial and ileal preparations. The pA_2 values are shown in Table 2.

Atropine, pirenzepine and the quaternary compounds *4a/4b* and *6a/6b* exhibited similar affinities for muscarinic receptors in ileum and atria. At both receptors the following rank order of potencies was obtained: atropine > *4a/4b* ≥ *6a/6b* > pirenzepine.

The affinities obtained for compounds *1a/1b, 2a/2b, 3a/3b,* and *5a/5b* were significantly greater at muscarinic receptors present in the ileum than at receptors in the atria, and the following rank orders of potencies were obtained; atria: *2a/2b* > *5a/5b* ≥ *3a/3b* ≥ *1a/1b*; ileum: *2a/2b* > *3a/3b* ≥ *5a/5b* ≥ *1a/1b*.

In contrast, the pA_2 value obtained for methoctramine was much lower in the ileum compared to the affinity obtained in the atria.

Discussion

This study has investigated the differences in affinity of a series of compounds structurally related to hexahydro-diphenidol (*3a*) and hexahydro-sila-diphenidol (*3b*) at muscarinic receptors. The principal conclusions we draw from these experiments are: (1) the differences in affinity values exhibited by atropine, pirenzepine, methoctramine and compounds *1a/1b–6a/6b* and the different rank orders of anti-

Table 2 Antagonist affinities (pA_2 values) at ileal and atrial muscarinic receptors[a]

Antagonist	*Ileum*	*Atria*
1a Demethyl-hexocyclium	7.63 ± 0.03	6.51 ± 0.08
1b Demethyl-sila-hexocyclium	7.87 ± 0.04	6.65 ± 0.04
2a Hexocyclium	8.49 ± 0.04	7.75 ± 0.03
2b Sila-hexocyclium	8.78 ± 0.05	7.57 ± 0.04
3a Hexahydro-diphenidol	7.98 ± 0.02	6.71 ± 0.04
3b Hexahydro-sila-diphenidol	7.96 ± 0.04[b]	6.53 ± 0.05[b]
4a HHD methiodide	8.00 ± 0.03	7.93 ± 0.04
4b HHSiD methiodide	8.18 ± 0.04	7.99 ± 0.02
5a Procyclidine	7.75 ± 0.04[a]	6.76 ± 0.04[a]
5b Sila-procyclidine	8.04 ± 0.04[a]	6.92 ± 0.04[a]
6a Tricyclamol	7.92 ± 0.06[a]	7.65 ± 0.03[a]
6b Sila-tricyclamol	8.22 ± 0.05[a]	7.88 ± 0.02[a]
Atropine	8.85 ± 0.03	9.21 ± 0.04
Pirenzepine	6.88 ± 0.04[b]	6.82 ± 0.03[b]
Methoctramine	6.17 ± 0.05	7.69 ± 0.03

The numbers show the mean estimate of the value of $-\log$ affinity constant ($pA_2 \pm$ s.e.), $n = 9$–16 experiments. [a] Arecaidine propargyl ester was used as the agonist in all cases, except carbachol. [b] Data previously published by Lambrecht *et al.*, (1988a,b)

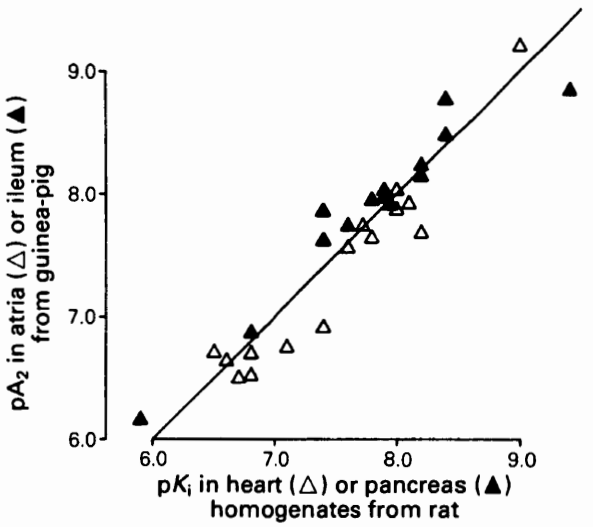

Figure 5 Comparison of pK_i and pA_2 values. pK_i values obtained in binding experiments are compared with pA_2 values obtained in pharmacological studies: data on rat cardiac homogenates are related to those on guinea-pig isolated atria (△), and rat pancreas homogenates with guinea-pig isolated ileum strips (▲) (data from Table 1 and 2).

muscarinic potencies confirm that NB-OK 1 cells, heart and pancreas possess different muscarinic receptors of the neuronal M_1, cardiac, and glandular/smooth muscle subtypes, respectively (Waelbroeck *et al.*, 1987a,b,c). (2) The rat heart and pancreas muscarinic binding sites are essentially similar to the muscarinic receptors mediating negative inotropic responses in guinea-pig atria and contractions in guina-pig ileum, respectively. (3) Structural variations in the hexahydro-diphenidol (*3a*) and hexahydro-sila-diphenidol (*3b*) molecules lead to new muscarinic antagonists which exhibit a different spectrum of selectivity from the parent compounds.

Comparison of binding and pharmacological experiments

Atropine, pirenzepine, methoctramine and compounds *1a/1b–6a/6b* showed quite wide variations in their affinities for the muscarinic receptors in pancreas, ileum and heart, their pK_i (Table 1) and pA_2 (Table 2) values differing by three orders of magnitude. The affinities of the antagonists for muscarinic receptors in rat pancreas and in guinea-pig ileum were very similar both individually and in rank order. A highly significant correlation was found between these values (Figure 5). The affinities for muscarinic receptors in rat heart and guinea-pig atria were also strikingly similar (Figure 5), but they were different from those obtained in pancreas and ileum for most antagonists. This implies that the structural demands for the antagonists made by the muscarinic binding sites in pancreas and heart, respectively, are very similar to those made by the receptors in ileum and atria, respectively.

Structure-selectivity relationships

In the course of structure-activity relationship studies of compounds of the diphenidol type, it was found that replacement of the central carbinol carbon atom by silicon increased the affinity to muscarinic receptors in guinea-pig ileum and atria up to one order of magnitude (Tacke & Zilch, 1986; Tacke & Becker, 1987). In contrast, sila-substitution of *1a/6a* (→*1b/6b*) had little effect on the affinity in binding studies as well as in pharmacological experiments (Tables 1 and 2). This might be explained by differences in electronic and stereochemical properties: compounds of the diphenidol type carry two phenyl rings at the central C/Si atom, whereas in the case of *1a/1b–6a/6b* one phenyl ring is hydrogenated to a cyclohexyl ring system.

The tertiary amines demethyl-hexocyclium (*1a*), procyclidine (*5a*) and their silicon analogues (*1b*, *5b*)

are muscarinic antagonists that possess a different selectivity profile from the parent compounds hexahydro-diphenidol (*3a*) and hexahydro-sila-diphenidol (*3b*). The following affinity rank orders at M_1, cardiac and glandular/smooth muscle receptors were obtained; *3a/3b*: M_1 = glandular/smooth muscle > cardiac; *1a/1b*, *5a/5b*: M_1 > glandular/smooth muscle > cardiac.

N-methylation of the tertiary amines *1a/1b*, *3a/3b* and *5a/5b* increased the affinity for cardiac as well as for NB-OK 1 cell M_1 receptors by up to one log unit (Tables 1 and 2). This also holds true for *1a/1b* (→*2a/2b*) at glandular/smooth muscle receptors in ileum and pancreas, whereas the affinity for glandular/smooth muscle receptors of *3a/3b* and *5a/5b* was not changed or only poorly increased by N-methylation. Thus, the selectivity profiles of the quaternary compounds hexocyclium (*2a*) and sila-hexocyclium (*2b*) are identical to that of the tertiary amines *1a/1b* (M_1 > glandular/smooth muscle > cardiac), the affinities of *2a/2b* being higher by about one order of magnitude than those of the tertiary compounds *1a/1b* at the three subtypes. In contrast, N-methylation of *3a/3b* (→*4a/4b*) and *5a/5b* (→*6a/6b*) changed the receptor selectivity patterns of the tertiary amines. The following affinity rank order was obtained; *4a/4b*, *6a/6b*: M_1 > glandular/smooth muscle ≥ cardiac. Thus, *4a/4b* and *6a/6b* show a selectivity profile that is qualitatively similar to that of pirenzepine.

In conclusion, the present study shows that on the basis of the binding profiles of pirenzepine, methoctramine and compounds *1a/1b–6a/6b* the muscarinic receptors in NB-OK 1 cells, heart and pancreas can be clearly subdivided into three subtypes: M_1, cardiac and glandular/smooth muscle receptors. We have provided evidence that the muscarinic binding sites in the rat heart and pancreas are very similar to guinea-pig atria and ileum receptors, respectively, but different from each other. We have shown that the antimuscarinic potency and subtype selectivity of hexahydro-diphenidol (*3a*) and hexahydro-sila-diphenidol (*3b*) and of the analogues *1a/1b*, *2a/2b* and *4a/4b–6a/6b* depend on different structural parameters (e.g. tertiary or quaternary nitrogen). The M_1, cardiac and glandular/smooth muscle muscarinic receptors make different structural demands for the antagonists thus different receptor selectivity profiles were obtained. Compounds *1a/1b–6a/6b* will facilitate further investigation of muscarinic receptor heterogeneity.

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