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p-Fluoro-hexahydro-sila-difenidol: The first $M_{2\beta}$-selective muscarinic antagonist

Günter Lambrecht *, Roland Feifel, Bernhard Forth [1], Carsten Strohmann [1], Reinhold Tacke [1] and Ernst Mutschler

Department of Pharmacology, University of Frankfurt, D-6000 Frankfurt/M, and [1] Institute of Inorganic and Analytical Chemistry, Technical University of Braunschweig, D-3300 Braunschweig, Federal Republic of Germany

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Muscarinic receptors are taken to comprise at least three subtypes: M_1 (neuronal type), $M_{2\alpha}$ (cardiac type) and $M_{2\beta}$ (smooth muscle/glandular type) (for recent review, see Mutschler et al., 1987). This subclassification is based mainly on the different affinities of selective antagonists such as pirenzepine ($M_1 \gg M_{2\alpha} = M_{2\beta}$), methoctramine ($M_{2\alpha} > M_1 > M_{2\beta}$) and hexahydro-sila-difenidol (HHSiD) ($M_1 = M_{2\beta} \gg M_{2\alpha}$) (Eltze, 1988a,b; Melchiorre et al., 1987; Mutschler et al., 1987; Waelbroeck et al., 1988). We report here on the antimuscarinic profile of p-fluoro-hexahydro-sila-difenidol (p-F-HHSiD; cyclohexyl(4-fluorophenyl)(3-piperidinopropyl)silanol) which exhibits a spectrum of selectivity different from that of the parent compound, HHSiD. To the best of our knowledge, p-F-HHSiD is the first muscarinic antagonist that shows $M_{2\beta}$-receptor selectivity.

The affinities of HHSiD, p-F-HHSiD and pirenzepine to muscarinic receptors were determined in rabbit vas deferens (M_1) and guinea-pig atria ($M_{2\alpha}$) and ileum ($M_{2\beta}$). Experiments on vasa deferentia were performed as described by Eltze (1988a,b). Briefly, male New Zealand white rabbits were killed by i.v. injection of pentobarbital sodium (120 mg/kg). The vasa deferentia were isolated, divided into four segments and suspended under 0.75 g tension in separate organ baths. The bathing fluid (31°C) was oxygenated (5% CO_2-95% O_2) and contained (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, KH_2PO_4 1.2, $NaHCO_3$ 25.0, (+)-glucose 11.1; yohimbine 1 μM. Twitch contractions were elicited by electrical field stimulation (0.05 Hz, 0.5 ms, 30 V). These effects were dose dependently inhibited by the M_1-selective agonist (4-((N-(4-chlorophenyl)carbamoyl)oxy)-2-butynyl)trimethylammonium iodide (4-Cl-McN-A-343) (Mutschler et al., 1987; Eltze, 1988a). Left atria and strips of ileal longitudinal muscle from adult guinea pigs were incubated under 0.5 g tension in oxygenated (5% CO_2-95% O_2) Tyrode solution (32°C; composition in mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, (+)-glucose 5.6). The atria were paced electrically (2 Hz, 3 ms, 5 V). The twitch contractions of vas deferens inhibited by 4-Cl-McN-A-343 ($pD_2 = 6.6$), the ileum contractions mediated by arecaidine propargyl ester ($pD_2 = 7.5$) and the negative inotropic responses in atria ($pD_2 = 8.1$) were recorded with a force-displacement transducer on multichannel recorders. Cumulative dose-response curves to the agonists were obtained before and after the addition of an antagonist (equilibration time: 30-60 min). At least three concentrations of antagonists were tested in the three tissues and the dose ratios for the antagonist-induced shifts in the agonist

* To whom all correspondence should be addressed: Department of Pharmacology, University of Frankfurt, Theodor-Stern-Kai 7, Gebäude 75A, D-6000 Frankfurt/M, F.R.G.

TABLE 1

Affinity profiles of pirenzepine, hexahydro-sila-difenidol (HHSiD) and p-fluoro-hexahydro-sila-difenidol (p-F-HHSiD) at muscarinic M_1-receptors in rabbit vas deferens and at M_2-receptors in guinea-pig ileum ($M_{2\beta}$) and atria ($M_{2\alpha}$). Dose-response curves for 4-Cl-McN-A-343 (vas deferens) and arecaidine propargyl ester (ileum and atria) were obtained and the dose ratios were calculated for the antagonist-induced parallel shifts of these curves. The pA_2 values were determined from Schild plots constrained to slope -1.0 since it was always verified that the experimental data generated regression lines with derived slopes not significantly different from unity. The results are presented as means ± S.E.M. for 12-15 experiments on each preparation. K_D ratios are given as a measure of receptor selectivity. These values were calculated from the antilog of the difference between respective pA_2 values.

	pA_2			Selectivity ratios		
	Vas deferens M_1	Ileum $M_{2\beta}$	Atria $M_{2\alpha}$	$M_1/M_{2\alpha}$	$M_{2\beta}/M_1$	$M_{2\beta}/M_{2\alpha}$
HHSiD	7.92 ± 0.07	7.96 ± 0.03	6.53 ± 0.05	25	1.1	27
p-F-HHSiD	6.68 ± 0.03	7.84 ± 0.03	6.01 ± 0.06	4.7	14.4	67
Pirenzepine	8.24 ± 0.06	6.88 ± 0.04	6.82 ± 0.03	26	0.04	1.2

dose-response curves were estimated. Schild plots were made with the linear regression by the method of least squares. The slopes of these plots were not significantly different from unity. The pA_2 values (table 1) were thus estimated by fitting the best straight line with a slope equal to unity.

Pirenzepine, HHSiD and p-F-HHSiD proved to be competitive antagonists at the three muscarinic receptor subtypes. The affinities of pirenzepine and HHSiD were very similar to those found in binding studies (Waelbroeck et al., 1988) and were consistent with the known receptor selectivities of these reference drugs (table 1). A different selectivity pattern was observed for p-F-HHSiD: $M_{2\beta} > M_1 > M_{2\alpha}$. p-F-HHSiD exhibited a high affinity ($pA_2 = 7.84$) for smooth muscle $M_{2\beta}$-receptors while its antimuscarinic potency at M_1- and $M_{2\alpha}$-receptors was lower by factors of 14.4 and 67, respectively. In addition, the receptor selectivity ratios found for p-F-HHSiD (table 1) were different from those obtained for pirenzepine and HHSiD at the three subtypes.

In conclusion, p-fluoro-hexahydro-sila-difenidol is the first $M_{2\beta}$-selective muscarinic antagonist. It has a selectivity for $M_{2\beta}$-receptors comparable to that of pirenzepine for M_1- and methoctramine for $M_{2\alpha}$-receptors. p-F-HHSiD can be used successfully to characterize M_2 muscarinic receptors directly. It will facilitate further investigation of muscarinic receptor heterogeneity.

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