European Journal of Pharmacology, 151 (1988) 155–156
Elsevier

EJP 0130R

Rapid communication

o-Methoxy-sila-hexocyclium: a new quaternary M_1-selective muscarinic antagonist

Günter Lambrecht *, Gernot Gmelin [1], Klaus Rafeiner [2], Carsten Strohmann [2], Reinhold Tacke [2] and Ernst Mutschler

Department of Pharmacology, University of Frankfurt, D-6000 Frankfurt/M, F.R.G., [1] Sandoz Ltd., CH-4002 Basel, Switzerland, and [2] Institute of Inorganic and Analytical Chemistry, Technical University of Braunschweig, D-3300 Braunschweig, F.R.G.

Received 17 May 1988, accepted 19 May 1988

The muscarinic receptors are currently divided into at least three subtypes: M_1, $M_{2\alpha}$ and $M_{2\beta}$ (for recent reviews, see Eglen and Whiting, 1986; Mutschler et al., 1987; 1988). M_1-Receptors are found in high density in neuronal tissues such as autonomic ganglia, cerebral cortex and hippocampus, whereas M_2-receptors are mainly present in lower brain areas and in peripheral effector organs such as heart ($M_{2\alpha}$) and smooth muscles ($M_{2\beta}$) as well as glands ($M_{2\beta}$). Their different affinities for selective muscarinic antagonists such as pirenzepine ($M_1 \gg M_{2\alpha} = M_{2\beta}$; Hammer and Giachetti, 1982; Mutschler et al., 1987; 1988) and methoctramine ($M_{2\alpha} > M_1 > M_{2\beta}$; Mutschler et al., 1988) are a major characteristic of these subtypes. Recent structure-selectivity relationship studies of a series of difenidol and hexocyclium analogues have led to the discovery of novel muscarinic antagonists, hexahydro-sila-difenidol ($M_1 = M_{2\beta} \gg M_{2\alpha}$) and sila-hexocyclium (SiHC; $M_1 \simeq M_{2\beta} > M_{2\alpha}$) (Mutschler et al., 1987; 1988), which display a high selectivity in blocking smooth muscle and glandular $M_{2\beta}$-receptors in comparison to cardiac $M_{2\alpha}$-receptors. We report here on the unique pharmacological profile of o-methoxy-sila-hexocyclium (o-MeSiHC; 4-{[cyclohexylhydroxy(2-methoxyphenyl)silyl]methyl}-1,1-dimethylpiperazinium methyl sulfate) which exhibits a spectrum of selectivity different from that of the parent compound (SiHC). To the best of our knowledge, o-MeSiHC is the first quaternary antimuscarinic agent which shows high M_1-receptor selectivity.

Experiments on ganglia were performed as described by Brown et al. (1980). Briefly, superior cervical ganglia were isolated from rats anaesthetised with urethane. The ganglia were suspended in separate heated chambers (36°C) and superfused with oxygenated (5% CO_2, 95% O_2) Krebs solution (composition in mmol/l: NaCl 124; KCl 3; $NaHCO_3$ 26; NaH_2PO_4 1.25; $CaCl_2$ 2; $MgCl_2$ 2; (+)-glucose 10). Muscarine-induced ($pD_2 = 7.4$) depolarisation was recorded differentially, via calomel electrodes, between the ganglion and its postganglionic trunk. Semicumulative dose-response curves for muscarine were obtained and the antagonists pirenzepine and o-MeSiHC were pre-equilibrated for 30 min. Left atria and strips of ileal longitudinal muscle from adult guinea-pigs were incubated under 0.5 g tension in 6 ml oxygenated (5% CO_2, 95% O_2) Tyrode solution (32°C; composition in mmol/l: 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 at 120 beats/min. The ileum contractions and negative inotropic responses of the atria were recorded with a force-displacement

* 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

Comparison of the pA_2 values for pirenzepine (Pz) and o-methoxy-sila-hexocyclium (o-MeSiHC) at muscarinic M_1-receptors of rat superior cervical ganglia and at M_2-receptors of guinea-pig ileum ($M_{2\beta}$) and atria ($M_{2\alpha}$). Dose-response curves to muscarine (ganglia) and arecaidine propargyl ester (ileum and atria) were obtained and dose ratios were estimated for antagonist-induced parallel shifts in these curves. pA_2 values were determined from Schild plots constrained to slope −1.0. The experimental data generated regression lines whose derived slopes were not significantly different from unity. The results are presented as means ± S.E.M. of 9-12 experiments on each preparation.

	pA_2		
	Ganglia	Ileum	Atria
Pz	8.30 ± 0.05	6.88 ± 0.04	6.82 ± 0.03
o-MeSiHC	8.31 ± 0.06	6.96 ± 0.03	6.41 ± 0.01

transducer on a Helcoscriptor. Cumulative dose-response curves to arecaidine propargyl ester (pD_2-atria = 8.1; pD_2-ileum = 7.5) (Mutschler et al., 1988) were obtained before and after the addition of an antagonist which was allowed to equilibrate for 30 min. At least three concentrations of antagonists were tested in the three tissues and dose ratios were estimated for antagonist-induced parallel shifts in the agonist dose-response curves. Schild plots were made, using 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 estimated by fitting to the data the best straight line with a slope equal to unity.

Pirenzepine and o-MeSiHC proved to be competitive antagonists at ganglionic M_1-, cardiac $M_{2\alpha}$- and ileal $M_{2\beta}$-receptors. Different selectivity patterns were observed; pirenzepine: $M_1 \gg M_{2\alpha} = M_{2\beta}$, o-MeSiHC: $M_1 \gg M_{2\beta} > M_{2\alpha}$. A 24-fold difference in pA_2 value was determined for both antagonists on the rat superior cervical ganglion (8.3. This value was very close to the pA_2 value of 8.36 for pirenzepine determined by Brown et al. (1980)) compared with the ileum (6.9). Pirenzepine exhibited similar affinities for cardiac $M_{2\alpha}$- and ileal $M_{2\beta}$-receptors. In contrast, the affinity obtained for o-MeSiHC was significantly lower at $M_{2\alpha}$-receptors in atria than at $M_{2\beta}$-receptors in the ileum. Thus, the selectivity of pirenzepine and o-MeSiHC for M_1- over $M_{2\alpha}$-receptors is 30- and 78-fold, respectively.

In conclusion, o-MeSiHC is a potent M_1-selective muscarinic antagonist and could be a useful new tool to further investigate muscarinic receptor heterogeneity. o-MeSiHC is the first quaternary ammonium compound with high M_1-receptor selectivity. It has the advantage over pirenzepine that its selectivity for M_1- over $M_{2\alpha}$-receptors is higher.

References

Brown, D.A., A. Forward and S. Marsh, 1980, Antagonist discrimination between ganglionic and ileal muscarinic receptors, Br. J. Pharmacol. 71, 362.

Eglen, R.M. and R.L. Whiting, 1986, Muscarinic receptor subtypes: a critique of the current classification and a proposal for a working nomenclature, J. Auton. Pharmacol. 5, 323.

Hammer, R. and A. Giachetti, 1982, Muscarinic receptor subtypes: M_1 and M_2. Biochemical and functional characterization, Life Sci. 31, 2991.

Mutschler, E., G. Gmelin, U. Moser, J. Wess and G. Lambrecht, 1987, Structure-activity relationships of drugs acting selectively at different muscarinic receptors, in: Pharmacology, International Congress Series No. 750, eds. M.J. Rand and C. Raper (Elsevier Science Publishers B.V., Amsterdam) p. 67.

Mutschler, E., U. Moser, J. Wess and G. Lambrecht, 1988, New approaches to the subclassification of muscarinic receptors, in: Recent Advances in Receptor Chemistry, eds. C. Melchiorre and M. Gianella (Elsevier Science Publishers B.V., Amsterdam) p. 195.