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Hexocyclium derivatives with a high selectivity for smooth muscle muscarinic receptors

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1 The affinity of a number of derivatives of the muscarinic antagonist, hexocyclium, containing an amidine cationic head, for guinea-pig cardiac and ileal receptors was investigated.

2 All the compounds studied displayed a greater affinity for muscular than for cardiac muscarinic receptors.

3 The 5 fold ileal selectivity of hexocyclium was increased by a number of chemical substitutions. The largest discrimination between receptors (about 200 fold) was found for the formamidine derivative.

4 The selectivity displayed by the hexocyclium derivatives stemmed from a greater decrease in affinity towards cardiac as compared to ileal receptors.

Introduction

The concept that cardiac and intestinal muscarinic receptors possess different pharmacological properties was first suggested by the preferential antagonism at cardiac receptors displayed by gallamine and related neuromuscular relaxants (Riker & Wescoe, 1951; Mitchelson, 1984). Subsequently, agents endowed with the reverse selectivity, i.e. greater affinity for smooth muscle receptors were also described, namely, 4-diphenylacetoxy-*N*-methyl piperidine methiodide (4-DAMP) (Barlow *et al.*, 1976) and analogues of procyclidine (sila-procyclidine and sila-diphenidol, Mutschler & Lambrecht, 1984). The discrimination afforded by either class of molecules ranges between 10 and 30 fold.

The good correlation between pharmacological and binding results, and the data from the cloned m2 receptors demonstrate a remarkable homogeneity of the cardiac receptor population (M_2 subtype; Hammer *et al.*, 1986; Giachetti *et al.*, 1986; Maeda *et al.*, 1988). However, evidence have been provided that at least two subtypes, M_2 and M_3 (glandular type), are present on visceral smooth muscle (Giraldo *et al.*, 1987; 1988; Maeda *et al.*, 1988). At present the relative contribution of each subtype to contractile function is still unclear, hampering the design of molecules with improved selectivity for muscular tissue.

While exploring the structural requirements for M_1 receptor antagonism, we found that replacement of the tertiary amino group of pirenzepine with a guanidine moiety did not affect M_1 affinity, but considerably reduced the affinity for M_2 receptors (Cereda *et al.*, 1989). It was therefore of interest to investigate the effect of the same substitution on receptors mediating smooth muscle contractility. Hexocyclium (Figure 1) was selected as a lead molecule since: (a) the compound is endowed with clinically relevant spasmolytic activity (Martindale, 1989) and (b) its sila-derivative shows some selectivity for smooth muscle receptors (Lambrecht *et al.*, 1988).

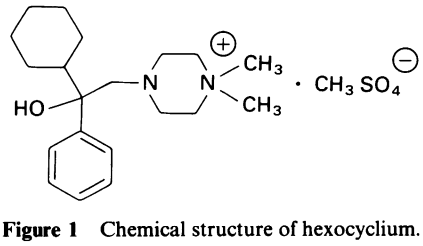

Figure 1 Chemical structure of hexocyclium.

This study describes the affinities for ileal and cardiac muscarinic receptors of derivatives of hexocyclium in which the onium head has been substituted by amidine-type groups, with the formamidine derivative, emerging as the most selective compound.

A preliminary account of these results has been given elsewhere (Donetti *et al.*, 1989).

Methods

Guinea-pig left atria

Tissues were mounted in McEwen's solution (mM: NaCl 131.6, KCl 5.6, $CaCl_2$ 2.16, $NaHCO_3$ 24.9, NaH_2PO_4 1.03, glucose 11 and sucrose 13) under 1 g tension, at 32°C, and stimulated through platinum electrodes (3 Hz, 2 ms, 100% above threshold voltage). Inotropic activity was recorded isometrically (Basile 7005 transducer, 7050 recorder).

Guinea-pig ileum

Segments of terminal ileum were suspended in Tyrode solution (mM: NaCl 137, KCl 2.68, $CaCl_2$ 1.82, $NaHCO_3$ 5.9, $MgCl_2$ 1.0, NaH_2PO_4 0.42 and glucose 5.6) at 37°C. Tension changes were recorded isotonically (Basile 7006 transducer, 7050 recorder).

Experimental procedure

In both preparations, muscarinic receptor stimulation was induced by cumulative additions of bethanechol. Antagonists were equilibrated for 60 min. Affinity measurements were estimated by either Schild analysis, fitting linear regression by least squares and verifying parallelism before calculating dose-ratios, or when only 4 replicates were performed, from the relation:

$$pA_2 = -\log([\text{Antagonist}]/(DR - 1)).$$

Compounds

Bethanechol hydrochloride was purchased from Sigma Chemical Co. (St Louis, MO, USA).

Hexocyclium (4-(2-cyclohexyl-2-hydroxy-2-phenyl ethyl)-1, 1-dimethyl piperazinium methyl sulphate) was synthesized by

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the method described in U.S. Patent 2, 907,765 (1959). The amidine derivatives (compounds 1–7, Table 1) were prepared by reacting their secondary amine precursors with ethyl formimidate, ethyl acetimidate, or cyanamide as described in the European Patent Appl. (publication number 0309424, 1989). The formamidine (1, 4–7), acetamidine (2), and guanidine derivatives (3) were obtained in satisfactory yields.

Results

Hexocyclium was a competitive antagonist of bethanechol in both guinea-pig atria and ileum, with a 5 fold greater affinity for ileal receptors ($P < 0.05$; Table 1).

The three amidine derivatives were also able to antagonize the muscarinic responses investigated in a competitive fashion. As can be seen from the affinity constants (Table 1), interaction of compounds 1–3 with muscarinic receptors was negatively affected by the substitutions. The pA_2 values at the cardiac receptor were drastically decreased (120 (compound 1) to 479 fold (compound 3)). In contrast, the decrease in ileal pA_2 values was much smaller (55 fold (compound 3) to less than 3 fold (compound 1)). This resulted in a large improvement of selectivity; from the 5 fold selectivity ratio of hexocyclium to 204 fold for compound 1 (Table 1).

Further modifications of compound 1 led to compounds with comparable M_2 affinity (compounds 4–7, Table 1). In the case of the phenyl (7) and the branched alkyl (6) derivatives, the affinity for the ileal receptors was reduced to a similar extent. None of these further chemical manipulations afforded a better selectivity than that observed for compound 1.

Discussion

The present study shows that substitution of hexocyclium quaternary nitrogen by amidine moieties leads to antagonists selective for smooth muscle receptors.

The selectivity originates from a different decremental effect exerted by the amidine substitution on the affinity for ileal and cardiac receptors. For the most favourable substitution, formamidine (compound 1), a negligible effect on ileal affinity is accompanied by a pronounced loss in cardiac affinity, resulting in the greatest discrimination (204 fold) so far reported for any smooth muscle selective antagonists. This indicates that M_2 receptors possess more stringent requirements for recognition of antagonists of this type.

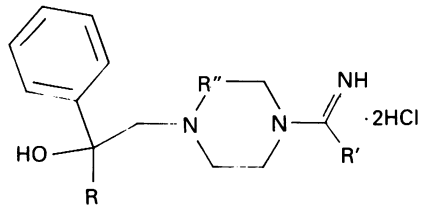
The physico-chemical features of amidines may account for the different decreases in affinity observed at the two subtypes. Owing to their pK_a values in the range 9–12 (Patai, 1975), amidine systems may be considered as structures intermediate between tertiary amines and quaternary onium compounds. They differ, however, from these latter moieties as tertiary amines and onium cationic heads possess a tetrahedral structure favouring van der Waals interactions (Belleau & Puranen, 1963), whereas amidinium cations have a high capacity to form hydrogen bonds (Sapse & Massa, 1980) and prefer the planar conformation (Mazurek *et al.*, 1983).

Further information that can be obtained from present results relates to the role of M_2 receptors in ileal contraction and is derived from inspection of affinity constants of compound 1. The concentrations employed to estimate its ileal pA_2 (10 to 100 nM) were below the K_B (575 nM) found for M_2 receptors, and indicate that this latter subtype plays a minor role in smooth muscle contraction. This supports previous similar conclusions obtained with M_2 selective compounds (Giraldo *et al.*, 1987; Roffel *et al.*, 1988; Ladinsky *et al.*, 1988).

A loss in M_2 affinity for amidine-substituted compounds is a feature found for one other type of muscarinic antagonist. Thus, for the guanyl derivative of pirenzepine, an M_1/M_2 ratio of 250 was found as a consequence of its decreased M_2 affinity (Cereda *et al.*, 1989). This suggests that amidine substituents on different classes of selective muscarinic antagonists are worth exploiting.

In conclusion, replacement of the quaternary head in the hexocyclium molecule by the amidine substituents, formamidine (1), acetamidine (2), and guanidine (3) affects affinities for ileal and cardiac receptors to a different extent, the overall result being an enhancement of smooth muscle selectivity. The greatest selectivity is found for the formamidine derivative and could not be improved by further variations of the molecular skeleton.

Table 1 General formula and affinity estimates (pA_2) for ileal and cardiac muscarinic receptors of amidine derivatives of hexocyclium



Compound	R	R′	R″	*MP* (°C)	*Ileum* ($pA_2 \pm$ s.e.)	*Atrium* ($pA_2 \pm$ s.e.)	*Selectivity ratio*
1	cyclohexyl	H	CH_2	218–220	8.56 ± 0.13 ($n = 11$) (0.96 ± 0.11)	6.25 ± 0.11 ($n = 11$) (1.30 ± 0.16)	204
2	cyclohexyl	CH_3	CH_2	201–202	7.68 ± 0.14 ($n = 9$) (1.09 ± 0.12)	6.00 ± 0.15 ($n = 15$) (1.04 ± 0.12)	48
3	cyclohexyl	NH_2	CH_2	183–185	7.27 ± 0.17 ($n = 9$) (0.97 ± 0.19)	5.65 ± 0.07 ($n = 9$) (1.22 ± 0.12)	42
4	cyclohexyl	H	CH_2CH_2	230–232	8.12 ± 0.29 ($n = 12$) (0.97 ± 0.24)	6.38 ± 0.04 ($n = 9$) (0.97 ± 0.04)	55
5	cyclopentyl	H	CH_2	>275	8.10 ± 0.11 ($n = 4$)	6.68 ± 0.15 ($n = 4$)	26
6	3-pentyl	H	CH_2	223–225	7.79 ± 0.12 ($n = 4$)	6.18 ± 0.02 ($n = 4$)	41
7	phenyl	H	CH_2	267–270	7.42 ± 0.06 ($n = 4$)	6.10 ± 0.02 ($n = 4$)	21
Hexocyclium					9.01 ± 0.28 ($n = 12$) (0.93 ± 0.19)	8.33 ± 0.27 ($n = 10$) (0.80 ± 0.16)	5

Compound 1 as monohydrochloride; for compounds 5–7 values are means ± s.e. (see methods); n = number of replicates; in parentheses slope ± s.e.

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