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Comparison of the affinity constant of some muscarinic receptor antagonists with their displacement of [^{3}H]quinuclidinyl benzilate binding in atrial and ileal longitudinal muscle of the guinea-pig

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The ability of the muscarinic receptor antagonists fenipramide, 4-diphenylacetoxy-*N*-methyl piperidine methiodide (4-DAMP) and secoverine to displace [^{3}H]QNB binding was correlated with the inhibition of responses of cholinomimetics at muscarinic receptors in the atria and ileal longitudinal muscle of the guinea-pig. Fenipramide and 4-DAMP exhibited a 2–4 fold higher affinity for muscarinic receptors in ileal longitudinal muscle in both types of experiments. Secoverine exhibited no difference in affinity in the two tissues.

Claims of heterogeneity amongst muscarinic receptors have been based partly on receptor binding studies using radiolabelled muscarinic receptor antagonists such as [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) (Ellis & Hoss 1982; Dunlap & Brown 1983) or [^{3}H]*N*-methylscopolamine (Hammer et al 1980; Stockton et al 1983) and partly on studies in-vivo or in-vitro measuring the relative potency or affinity of muscarinic receptor antagonists (Brown et al 1980; Hammer & Giachetti 1982). To determine whether there was a correlation in the two types of experiments, a comparison was made of the affinities of some muscarinic receptor antagonists determined in isolated tissue experiments in guinea-pig atria and ileal longitudinal muscle with the ability of these antagonists to displace [^{3}H]QNB binding in the tissues.

The muscarinic receptor antagonists investigated were 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP), secoverine, fenipramide (HO 9980, α,α-diphenyl-γ-piperidyl butyramide HCl) and atropine. 4-DAMP has been reported to possess a higher affinity for muscarinic receptors in ileum than in atria (Barlow et al 1976, 1980). Secoverine also possesses selectivity for some muscarinic receptors as it blocks the receptors in ileum in lower doses than those required in salivary or lachrymal glands (Zwagemakers & Claassen 1980, 1981). Fenipramide was originally investigated by Schaumann & Lindner (1951) and shown to possess potent muscarinic receptor blocking activity with minimal antispasmodic activity. Its structure is related to that of difenidol, a compound which exhibits some selectivity for ileal muscarinic receptors over those in atria (Mutschler & Lambrecht 1984).

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Methods and materials

Concentration-response curves for the negative inotropic, negative chronotropic or contractile responses to carbachol or arecaidine propargyl ester were obtained in guinea-pig left atrium (driven at 3 Hz), right atrium (spontaneously beating) or ileal longitudinal muscle respectively. Tissues were bathed in McEwen's solution (McEwen 1956) at 37 °C and gassed with 95% oxygen and 5% carbon dioxide. Responses in duplicate were recorded isometrically under a resting tension of 0·5 g. Dose-ratios were calculated from the concentration giving 50% of the maximal responses before and after 40 min (fenipramide, secoverine) or 60 min (atropine, 4-DAMP) incubation with the antagonists. Mean pK_B values were estimated from Arunlakshana-Schild (A–S) plots when several concentrations of antagonist were employed. When only one concentration of antagonist [B] was used, a mean 'pA_2' value was estimated from the dose-ratio (DR) obtained, using the relation:

$$pA_2 = \log (DR - 1) - \log [B]$$

Table 1. Comparison of the dissociation constants for the muscarinic receptor antagonists in guinea-pig left atrium driven at 3 Hz and ileal longitudinal muscle using carbachol as agonist.

	Left atrium		Ileal longitudinal muscle		
Drug	pK_B	Slope*	pK_B	Slope*	Δ
Fenipramide	8·47 ± 0·07	1·02 ± 0·07 (36)	8·89 ± 0·09	1·14 ± 0·09 (25)	2·63†
4-DAMP	8·45 ± 0·08	0·98 ± 0·08 (15)	9·07 ± 0·12	1·00 ± 0·09 (22)	4·17†
Secoverine‡	8·22 ± 0·14	0·93 ± 0·09 (15)	8·17 ± 0·10	0·97 ± 0·07 (11)	0·89
Atropine§	9·19 ± 0·16 (7)	—	9·11 ± 0·09 (4)	—	0·83

* Mean slope ± s.e.m. (number of data points) of the A–S plot. None of the slopes were significantly different from unity ($P > 0·05$).

† Comparison of mean pK_B values from left atrium and ileal longitudinal muscle (*t*-test). For fenipramide $P < 0·05$, for 4-DAMP $P < 0·01$.

‡ Data from Choo & Mitchelson (1985).

§ 'pA_2' values based on geometric mean dose-ratios (±s.e.m., number of experiments) obtained with atropine (10 nM).

The effect of antagonists on the binding of the specific muscarinic ligand [^{3}H](−)QNB (80 pM) was investigated in homogenates of atrial pairs or ileal longitudinal muscle in 50 mM phosphate buffer (pH 7·4) at 37 °C. Incubations were over 60 min and terminated by the rapid filtration technique using HAWP 02500 filters as previously described (Choo et al 1985). Data was analysed by the non-linear curve-fitting programme LIGAND (Munson & Rodbard 1980).

The following drugs were used: arecaidine propargyl ester (gift, courtesy Dr G. Lambrecht), atropine sulphate (Sigma), carbachol (Sigma), 4-diphenylacetoxy-*N*-methylpiperidine methiodide (gift, courtesy Dr R. B. Barlow), fenipramide hydrochloride (Hoechst), [^{3}H](−)-quinuclidinyl benzilate (New England Nuclear: specific activity 33–40 Ci mmol^{-1}), secoverine hydrochloride (Duphar).

Results and discussion

Fenipramide was initially reported to be a potent muscarinic receptor antagonist with a similar potency to atropine for spasmolytic activity against non-cholinergic agonists in the ileum (Schaumann & Lindner 1951). In concentrations of 5 nM to 0·1 μM it competitively inhibited negative inotropic responses in left atrium and contractile responses in ileal longitudinal muscle produced by carbachol (Table 1). Negative chronotropic responses to carbachol in the right atrium were inhibited competitively to the same extent as the left atrial responses ($P > 0·05$). The mean pK_B value was 8·60 ± 0·12 (slope of A–S plot; 0·95 ± 0·12 (12 data points)). However, there was a significant difference ($P < 0·05$) between the mean pK_B values obtained in the left atrium and ileal longitudinal muscle with a ca three-fold higher affinity for muscarinic receptors in the ileum. Constraining the slopes of the A–S plots to unity (Mackay 1978) gave pK_B values of 8·49 ± 0·08 (left atrium), 8·56 ± 0·13 (right atrium) and 9·10 ± 0·10 (ileal longitudinal muscle). Experiments conducted with arecaidine propargyl ester, a cholinomimetic with high selectivity for muscarinic receptors (Mutschler & Lambrecht 1984), also produced significantly higher ($P < 0·01$) pA_2 values for fenipramide (0·1 μM) in ileum (9·02 ± 0·05; 3) (mean ± s.e.m., number of experiments) than in left atrium (8·69 ± 0·02; 3).

The degree of selectivity of fenipramide was comparable to that obtained with 4-DAMP (0·01–1 μM) (Table 1), another compound previously reported to exhibit selectivity for ileal muscarinic receptors (Barlow et al 1976, 1980). The corresponding pK_B values of 4-DAMP with the slope constrained to unity were 8·43 ± 0·10 (left atrium) and 9·08 ± 0·10 (ileal longitudinal muscle).

Secoverine was reported to be a potent antagonist at muscarinic receptors in the gastrointestinal tract but appeared less active at other muscarinic receptor sites in various glands and in the eye (Zwagemakers & Claassen 1980, 1981). In the present investigation, secoverine (10 nM–1 μM) like atropine, exhibited a similar affinity for atrial muscarinic receptors as for those in ileal longitudinal muscle (Table 1). Constraining the slopes of the A–S plots for secoverine to unity, gave corresponding pK_B values of 8·10 ± 0·22 (left atrium) and 8·13 ± 0·25 (ileal longitudinal muscle).

In binding experiments, fenipramide, 4-DAMP and secoverine displaced [^{3}H]QNB binding in both atrial and ileal tissue. The pK_I values obtained in ileal longitudinal muscle were higher than those in atria and the difference was significant for both fenipramide ($P < 0·01$) and 4-DAMP ($P < 0·01$) (Table 2). In contrast, secoverine displaced [^{3}H]QNB binding to a similar extent in both tissues ($P > 0·05$) (Table 2).

The finding that fenipramide exhibited selectivity for muscarinic receptors in the ileum supports previous evidence indicating a heterogeneity of muscarinic receptors in atrial and ileal tissue (Marshall et al 1980; Mutschler & Lambrecht 1984). The degree of selectivity for ileal muscarinic receptors obtained with fenipramide although significant, was not as marked as that reported for the silicon analogue of procyclidine (38-fold) which appeared to be the most selective compound from the group of compounds related to procyclidine and difenidol investigated by Lambrecht et al (1982) and Mutschler & Lambrecht (1984). Secoverine, like piren-

Table 2. Comparison of the ability of fenipramide, 4-DAMP and secoverine to displace [^{3}H]QNB binding in homogenates of guinea-pig atria and ileal longitudinal muscle.

	Atria		Ileal longitudinal muscle		
Drug	K_I (n) (95% C.L.)‡ nM	pK_I (±s.e.m.)	K_I (n) (95% C.L.)‡ nM	pK_I (±s.e.m.)	Δ
Fenipramide	13·5 (4) (7·0–26·1)	7·87 ± 0·09	3·9 (4) (2·2–7·0)	8·41 ± 0·08	3·46†
4-DAMP	25·2 (7) (17·1–37·0)	7·60 ± 0·07	9·7 (7) (5·9–16·0)	8·01 ± 0·09	2·59†
Secoverine	5·1 (4) (4·5–5·9)	8·29 ± 0·02	4·3 (4) (2·5–7·2)	8·37 ± 0·07	1·21
Atropine*	2·8	8·6	1·9	8·7	1·5

* Data from Choo et al (1985).
† Difference is significant ($P < 0·01$).
‡ (95% C.L.) = 95% confidence limits.

zepine (Barlow et al 1981; Fuder 1982; Fuder et al 1982) did not differentiate between ileal and atrial receptors although these two antagonists differentiated between various muscarinic receptors in other tissues in-vivo, suggesting that muscarinic receptors are heterogeneous, at least in their interaction with antagonists, and that it may be possible to exploit this therapeutically.

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