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The affinity of some selective muscarinic receptor antagonists for the muscarinic receptor mediating endothelial-dependent relaxation of the rabbit and rat thoracic aorta

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The pK_B values determined for pirenzepine, 4-DAMP, secoverine and gallamine against acetylcholine-mediated relaxant effects in rabbit aorta indicate that this muscarinic receptor closely resembles that which mediates contraction of non-vascular smooth muscle. The results of the present study argue against the presence of a novel type of muscarinic receptor mediating endothelium-dependent relaxation.

Pirenzepine has been used widely to characterize muscarinic receptors since it appears to interact with high affinity for some muscarinic receptors while at other muscarinic receptors it has a 20- to 50-fold lower affinity (Brown et al 1980; Hammer et al 1980; Hammer & Giachetti 1982; Wess et al 1984). Other selective muscarinic receptor antagonists can distinguish between sites exhibiting low affinity for pirenzepine. Gallamine inhibits muscarinic receptors on cardiac muscle at lower concentrations than required for muscarinic receptors on smooth muscle of the ileum and bladder (Riker & Wescoe 1951; Clark & Mitchelson 1976) while 4-DAMP (4-diphenylacetoxy-*N*-methylpiperidine methiodide) exhibits the reverse order of selectivity (Barlow et al 1976, 1980). Secoverine does not distinguish between muscarinic receptors in smooth and cardiac muscle (Choo & Mitchelson 1985) but shows a comparatively lower affinity for some glandular muscarinic receptors (Zwagemakers & Claassen 1980, 1981).

Using K_B values for atropine, the relative potencies of muscarinic agonists together with estimates of agonist K_A (dissociation constant) values, Furchgott & Cherry (1984) could find no evidence for the presence of a novel subtype of muscarinic receptor mediating relaxation in rabbit aortic ring preparations. However, a later study by Eglen & Whiting (1985) using a number of selective muscarinic receptor antagonists and in particular pirenzepine, concluded that the muscarinic receptor mediating relaxation differed from that observed in the central nervous system, heart and gastrointestinal tract. It was considered that one possible explanation of the findings by Eglen & Whiting (1985) was that pirenzepine exhibited a selective blockade of the muscarinic receptors subserving endothelial relaxation and had a lower affinity for the muscarinic receptors on the smooth muscle mediating contraction. This led to a reinvestigation of the effects of antagonists on the relaxant response to acetylcholine in the rabbit aorta together with an investigation in the rat aorta where contractile responses to muscarinic agonists are not apparent (Rapaport & Murad 1983).

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Methods

Isolated rings of rat or rabbit aorta were set up in McEwen's solution (McEwen 1956) at 37 °C under a resting tension of 2 g. Tissues were contracted with phenylephrine, 1 μM, a concentration giving ca 80% of the maximal response to the agonist. Reproducible, cumulative, concentration-response curves for relaxations produced by acetylcholine were obtained in the absence and presence of various antagonists. For all antagonists a 30 min equilibration was used except for atropine where the incubation time was 60 min.

Responses to acetylcholine were expressed as percentage inhibition of the contractile response to phenylephrine. Dose-ratios (DR) were calculated for each molar concentration of antagonist ([B]) using the respective EC50 values for acetylcholine in the absence and presence of the antagonist. For each concentration of antagonist, log (DR-1) values from individual experiments were plotted against log [B] with slope values calculated by linear regression analysis. For competitive antagonists (slope values not significantly different to unity) pK_B values were calculated by constraining the slope of the log (DR-1) vs log [B] plot (Schild or A-S plot) to unity (Mackay 1978).

Results

In rabbit aorta the geometric mean EC50 (95% confidence limits, n) for acetylcholine was 0·14 μM (0·13–0·16, 28). Physostigmine (0·1 μM, n = 3) did not alter the shape or position of the acetylcholine dose-response curves. All of the antagonists caused parallel rightward displacement of the concentration-response curves for acetylcholine without altering its maximal relaxant effect. Data obtained with the various antagonists is shown in Table 1. As the slopes of the Schild plots did not differ from unity, pK_B values as shown in the last column of Table 1 were estimated by constraining slopes of the plots to unity (Mackay 1978). Five control experiments carried out over the same time as those with the antagonists showed that the mean EC50 for acetylcholine did not alter significantly over 180 min.

Table 1. Data obtained from Schild plots using various antagonists on acetylcholine-induced relaxation of rabbit aorta.

Antagonist	Concn range	Slope of A-S plot log(DR-1) vs log [B] (data points)	X-axis-intercept (pA_2)	pK_B† value (± s.e.m.)
4-DAMP	3·0 – 30 nM	1·12 ± 0·19 (11)	8·95	9·07 ± 0·07
Gallamine	0·1 – 1 mM	0·58 ± 0·38 (10)	3·38	3·42 ± 0·13
Pirenzepine	0·3 – 3 μM	1·20 ± 0·10 (21)	6·75	6·88 ± 0·04
Secoverine	10·0 – 300 nM	1·10 ± 0·09 (11)	7·77	7·83 ± 0·04

† Slope of regression constrained to unity (Mackay 1978)

The geometric mean EC50 values (95% confidence limits) were 0·13 μM (0·08–0·19) initially and after 60 min; 0·13 μM (0·09–0·18), and 180 min; 0·11 μM (0·08–0·15).

Experiments with rat aorta produced the same mean pK_B value for pirenzepine (6·71 ± 0·11, n = 12 data points) provided that only one concentration of antagonist was investigated in each tissue. It was noted that when several concentrations of antagonist were used in the one preparation the resulting A-S plot had a low slope. This was not a general phenomenon as it did not occur when atropine was used at several concentrations in the same tissue (slope 1·00 ± 0·11, pK_B 8·86 ± 0·07; n = 9 data points).

In both tissues removal of the endothelium by rubbing the luminal surface gently with cotton wool or a wooden stick led to abolition of the relaxant response to acetylcholine. In some experiments the rabbit aorta, with endothelium removed, exhibited contractions to acetylcholine (0·3–100 μM) by stimulation of muscarinic receptors since responses were inhibited by atropine (0·01–0·1 μM) or pirenzepine (0·5 μM). However, in many experiments following removal of the endothelium with a wooden stick as used by Furchgott & Zawadzki (1980) and Martin et al (1985), the tissue only exhibited contractions at very high concentrations of acetylcholine (EC50 50–300 μM). These contractions were inhibited by hexamethonium (30 μM) and not by atropine (1 μM) indicating possible activation of nicotinic receptors on noradrenergic nerve endings (Furchgott et al 1981). As noted previously by Rapaport & Murad (1983) acetylcholine was devoid of contractile activity in endothelium-denuded preparations of rat aorta.

Discussion

Comparison of the pK_B values for pirenzepine, secoverine, 4-DAMP and gallamine against acetylcholine-mediated relaxation in rabbit aorta with previously reported values for these antagonists in other tissues, leads to the conclusion that the muscarinic receptors mediating relaxation of the aorta are similar to those mediating contraction of non-vascular smooth muscle and dissimilar to those that mediate negative chronotropic and inotropic effects in cardiac tissue (Barlow et al 1976, 1980; Mitchelson 1984; Choo & Mitchelson 1985). Thus, in intestinal tissue, pK_B values for pirenzepine range from 6·7–7·0, for secoverine from 8·1–8·3, for 4-DAMP from 8·7–9·1 and for gallamine from <3·0 to 4·7. For atria, affinity values for 4-DAMP are lower, and for gallamine higher, than the corresponding ileal values by at least 10-fold. With the exception of the value for pirenzepine, the pK_B values in our experiments are in agreement with those reported by Eglen & Whiting (1985). In the latter study the pA_2 value for pirenzepine using acetylcholine as agonist (7·57) was found to be higher than that observed in smooth muscle (6·7–7·0) but less than its reported value at high affinity sites in the nervous system (8·4, Brown et al 1980).

Initially, it was considered that post junctional muscarinic receptors mediating contraction of the rabbit aorta may have exhibited a lower affinity for pirenzepine, which would enhance inhibition of the relaxant response to acetylcholine and thus account for the high pA_2 value originally reported for pirenzepine. However, in our hands, the muscarinic receptor associated with relaxation of the rabbit aorta exhibited a similar low affinity toward pirenzepine as that in the rat aorta, where contractile responses to cholinomimetics are not present following removal of the endothelium (Rapaport & Murad 1983). Furthermore, in one experiment with rabbit aorta where a dose-ratio was determined for pirenzepine (0·5 μM) against contractile responses to acetylcholine, a pK_B value of 7·0 was estimated. In rings of dog coronary artery the pK_B value for pirenzepine against methacholine-induced relaxations has also been found to be 7·01 (T. M. Cocks & J. A. Angus, personal communication).

The only difference in methodology between that of Eglen & Whiting (1985) and the present study is that their experiments were performed by superfusing rather than bathing the tissues which we did in this study. Why the two techniques should not give identical results for pirenzepine, as was apparent for the other 3 antagonists, is not readily apparent.

In conclusion the results of the present study, based on the pK_B values of secoverine, 4-DAMP, gallamine and pirenzepine, clearly indicate that the muscarinic receptor mediating relaxation in the rabbit (and rat) aorta appeared to have similar characteristics to the muscarinic receptor in non-vascular smooth muscle.

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REFERENCES

Barlow, R. B., Berry, K. J., Glenton, P. A. M., Nikolaou, N. M., Soh, K. S. (1976) Br. J. Pharmacol. 58: 613–620

Barlow, R. B., Burston, K. N., Vis, A. (1980) Ibid. 68: 141P–142P

Brown, D. A., Forward, A., Marsh, S. (1980) Ibid. 71: 362–364

Choo, L.K., Mitchelson, F. J. (1985) Clin. Exp. Pharmacol. Physiol. 12: 95–98

Clark, A. L., Mitchelson, F. (1976) Br. J. Pharmacol. 58: 323–331

Eglen, R. M., Whiting, R. L. (1985) Ibid. 84: 3–5

Furchgott, R. F., Cherry, P. D. (1984) in: Hirschowitz, B. I., et al (eds) Trends in Pharmacol. Sci. Suppl: Subtypes of muscarinic receptors. Elsevier, Amsterdam, pp 45–48

Furchgott, R. F., Zawadzki, J. V. (1980) Nature 288: 373–376

Furchgott, R. F., Zawadzki, J. V., Cherry, P. D. (1981) in: Vanhoutte, P. M., Leusen, I. (eds) Vasodilatation. Raven Press, NY, pp 49–66

Hammer, R., Giachetti, A. (1982) Life Sci. 31: 2991–2998

Hammer, R., Berrie, C. P., Birdsall, N. J. M., Burgen, A. S. V., Hulme, E. C. (1980) Nature 283: 90–92

Mackay, D. (1978) J. Pharm. Pharmacol. 30: 312–314

Martin, W., Villani, G. M., Jothianandan, D., Furchgott, R. F. (1985) J. Pharmacol. Exp. Ther. 232: 708–716

McEwen, L. M. (1956) J. Physiol. (Lond.) 131: 678–689

Mitchelson, F. (1984) in: Hirschowitz, B. I., Hammer, R., Giachetti, A., Keirns, J. J., Levine, R. R. (eds) Trends Pharmacol. Sci. Suppl: Subtypes of muscarinic receptors. Elsevier, Amsterdam, pp 12–16

Rapaport, R. M., Murad, F. (1983) Circ. Res. 52: 352–357

Riker, W. F., Wescoe, W. C. (1951) Ann. N.Y. Acad. Sci. 54: 373–392

Wess, J., Lambrecht, G., Moser, V., Mutschler, E. (1984) Life Sci. 35: 553–560

Zwagemakers, J. M. A., Claassen, V. (1980) Arzneimittel-Forsch. 30: 1517–1526

Zwagemakers, J. M. A., Claassen, V. (1981) Eur. J. Pharmacol. 71: 165–168

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Luminal acid in stress ulceration and the antiulcer action of verapamil in rat stomachs

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The role of luminal acid and the influenee of the antisecretory action of verapamil in stress ulcer prevention in rat stomachs have been studied. Intraperitoneally injected verapamil, 4 mg kg^{-1}, inhibited gastric acid secretion and ulcer formation, however, a 2 mg kg^{-1} dose, which did not significantly influence acid output, also had an antiulcer effect. Intraperitoneal injection of bethanechol, 1·2 or 3·6 mg kg^{-1}, increased gastric acid output, but did not influence stress-induced ulcer formation. Oral administration of HCl, 25 or 50 μequiv, aggravated stress ulceration in a dose-dependent manner; this lesion-worsening effect was prevented by pretreatment with verapamil or bethanechol. The gastric luminal acid content in 2 h pylorus-ligated rats was similar in the groups given either bethanechol or HCl. These findings indicate that the antisecretory action of verapamil may not account for its antiulcer effect. It is suggested that endogenous and exogenous luminal acid may have different influences on stress ulcer formation.

Verapamil, a calcium channel blocker, has been shown to prevent stress ulceration (Ogle et al 1985a, b). Its antiulcer effects are thought to be due to inhibition of mast cell degranulation (Ogle et al 1985a) and of gastric motility (Ogle et al 1985b). However, the relationship of its antisecretory action to stress ulcer prevention remains undefined. Although the presence of acid seems to be a prerequisite for ulcer formation (Mersereau & Hinchey 1973; Ritchie 1975), there are reports indicating that gastric acid may play only a minor role in glandular mucosal lesion formation in stressed rats (Takagi & Okabe 1970; Cho & Ogle 1979). The present study examines the effects of luminal acid, elevated either by bethanechol injection or by oral administration of HCl, on stress-induced gastric ulceration. The possible role of the antisecretory action of verapamil in antagonizing stress-induced lesion formation in rat stomachs has also been evaluated.

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Methods

Female Sprague-Dawley rats (170–200 g) were reared on a balanced laboratory diet (Ralston Purina Co.) and given ordinary tap water to drink. They were housed in a room with controlled temperature (22 ± 1 °C) and humidity (65–70%). Animals were deprived of food for 48 h before use, but allowed free access to a solution of 8% w/v sucrose in 0·2% w/v NaCl (Cho & Ogle 1979) which was removed 1 h before experimentation.