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DOES PIRENZEPINE DISTINGUISH BETWEEN 'SUBTYPES' OF MUSCARINIC RECEPTORS?

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Pharmacological studies with pirenzepine were carried out on the isolated ileum and atrium of the guinea-pig and on the acid secretion from the isolated stomach of the mouse. Pirenzepine inhibited the bethanechol-evoked changes in all three organs in a dose-dependent manner. The slopes of the Schild-plots confirmed the competitive nature of the antagonism by pirenzepine. The estimated pA_2-values were very similar. Based on these data, it might be concluded that pirenzepine is an anticholinoceptor compound without specific affinity for gastric muscarinic receptors.

Introduction Pirenzepine, a tricyclic compound, has been proved effective in ulcer therapy (Blum & Hammer, 1979). Animal studies have shown that it inhibits gastric acid secretion stimulated by different secretagogues (Leitold & Engelhorn 1977). It has also been shown that the compound has some anticholinergic properties (Leitold, Engelhorn, Ballhouse, Kuhn & Ziegler, 1977). Recently, Hammer, Berrie, Birdsall, Burgen & Hulme (1980) have demonstrated that pirenzepine has different affinities for muscarinic receptors in several peripheral tissues. They concluded that pirenzepine is able to discriminate between different subclasses of muscarinic binding sites. The present paper is concerned with an investigation into whether pirenzepine is able to distinguish between the supposed subtypes of muscarinic receptors.

Methods

Guinea-pig isolated ileum Guinea-pigs weighing between 350–450 g were killed by cervical dislocation. The terminal ileum was removed immediately, washed and mounted in a 50 ml bath containing Tyrode solution, gassed with 95% O_2 plus 5% CO_2 and maintained at 37°C. The tissue was loaded with 0.5 g. Contractions were detected by a force transducer and registered on a pen-recorder.

Guinea-pig isolated atrium After killing the animal, a triangular piece of the right atrium (including the sino-atrial node) was quickly removed. This atrial strip was then mounted in a 35 ml bath containing McEwan solution at 34°C and gassed with 95% O_2 plus 5% CO_2. Strips were pre-loaded with 1.0 g. The frequency was recorded continuously via a transducer on a recorder.

Isolated stomach of the mouse The isolated stomach preparation has been described elsewhere (Szelenyi, 1981). Briefly, the stomach was exposed, cannulated at the pyloric sphincter and in the fore-stomach, washed and then rapidly dissected out. It was placed in a 40 ml bath containing modified Krebs-Henseleit solution gassed vigorously with 95% O_2 plus 5% CO_2 at 37°C. The lumen of the stomach was perfused at a rate of 1 ml/min. The H^+-ion concentration was continuously recorded in the effluent perfusate by means of a pH-electrode connected via a pH-meter to a pen-recorder. The rate of acid secretion was expressed as nmol/min.

Analysis of results Dose-ratios were calculated from a 2 + 2 assay (Colquhoun, 1971). A plot of log (dose-ratio − 1) on log (antagonist concentration) was also calculated in order to establish apparent pA_2-values and the nature of antagonism (Arunlakshana & Schild, 1959).

Drugs The following drugs were used in this study: histamine acid phosphate, bethanechol (Sigma Chemical Co., Taufkirchen), atropine sulphate (Merck AG, Darmstadt) and pirenzepine dihydrochloride (Gastrozepin, Dr Karl Thomae, Biberach).

Results The results are summarized in Table 1.

Bethanechol-evoked contractions of the guinea-pig isolated ileum were inhibited by atropine and pirenzepine in a concentration-dependent manner. As can be seen from Table 1, the pA_2-values for atropine and pirenzepine were significantly different from each other. Pirenzepine had approximately 1/100th the potency of atropine. The slopes of the so-called Schild-plots were not different from unity, indicating that the antagonism was of a competitive nature.

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Table 1 The antimuscarinic activity of atropine and pirenzepine on bethanechol-induced changes investigated on different organs (mean with 95% confidence limits)

Organ	*Ileum*		*Atrium*		*Stomach*	
Parameter	*Muscle contraction*		*Pacemaker activity*		*Acid secretion*	
Antagonist	pA_2	slope	pA_2	slope	pA_2	slope
Atropine	8.72	1.07	8.46	0.97	7.78	1.03
	(8.61–8.87)	±0.19	(8.28–8.71)	±0.11	(7.67–7.95)	±0.06
Pirenzepine	6.66	1.12	6.45	0.85	6.32	1.08
	(6.41–7.04)	±0.25	(6.23–6.71)	±0.23	(6.08–6.52)	±0.18

Cumulative dose-response curves to bethanechol were also established on the isolated atrium both in the presence and in the absence of the two compounds. Pirenzepine and atropine inhibited the bethanechol-induced decrease in the rate of the beating of the atrium in a concentration-related manner. The calculated pA_2 value for pirenzepine was 6.45 and for atropine 8.46. Thus, pirenzepine had approximately 1/100th the potency of atropine in inhibiting the action of bethanechol on the guinea-pig isolated atrium. Linear regressions were obtained with slopes of 0.85 and 0.97, respectively, suggesting that the antagonism on this organ also was of a competitive nature.

The effect of pirenzepine and atropine on acid secretion was studied on the isolated stomach of the mouse. Neither atropine nor pirenzepine influenced the basal rate of acid secretion. In the presence of either atropine or pirenzepine, dose-response curves to bethanechol were shifted to the right without depression of the maximum response. The pA_2 value for pirenzepine was 6.32 and for atropine 7.35. Thus, on this model, pirenzepine had approximately 1/10th the potency of atropine. The fact that the slopes of the Schild-plots were not different from unity suggests that the antagonism was of a competitive nature.

Pirenzepine did not influence acid secretion induced by histamine up to a concentration of 10^{-4} mol/l.

Discussion In the present study, the anticholinoceptor activity of pirenzepine has been confirmed under *in vitro* conditions in different isolated organs. The estimated pA_2 values for pirenzepine were very similar to each other. With regard to the classical definition of the pA_2 and its interpretation in the involvement of the receptors (Arunlakshana & Schild, 1959), there is no evidence that pirenzepine discriminates among subtypes of muscarinic receptors, at least, in these organs. It is probable that muscarinic receptors located on the parietal cells are not different from other muscarinic receptors. The pA_2 value for atropine estimated on the bethanechol-induced acid secretion was about 10 fold lower than those calculated on the ileum or the atrium. As shown in an earlier paper (Szelenyi & Vergin, 1980), there is no loss of atropine on the luminal side of the isolated stomach, which might explain the apparently lower pA_2 value for atropine. Angus & Black (1979) demonstrated that local tissue factors (diffusion, uptake, etc.) in the perfused stomach preparation are responsible for the observed deviation on the estimated pA_2 values. Of course, this possibility cannot be excluded for pirenzepine. However, there is some evidence that the gastric serosal barrier can be more easily overcome by pirenzepine that by atropine. Main & Pearce (1981) found a pA_2 value of 6.80 for pirenzepine in their gastric mucosa preparation which was free of serosa and thus, of penetration difficulties. In anaesthetized rats with perfused stomachs, pirenzepine also had approximately 1/100th the potency of atropine in blocking carbachol-stimulated secretion, as found by Parsons, Bunce, Blakemore & Rasmussen (1979).

From the results, it seems clear that the actions of pirenzepine can simply be explained by its antimuscarinic properties. It is not likely that pirenzepine differentiates between subtypes of muscarinic receptors located on parietal cells, since our results, the uniform pA_2 values obtained on different organs and their agreement with previous results (Parsons *et al.*, 1979; Main & Pearce, 1981), point to a homogeneous receptor population. In our opinion, pirenzepine is an antimuscarinic drug with a lower affinity for the receptor than atropine. In agreement with our present results, Daly, Humphray & Stables (1982) have recently demonstrated that inhibitory doses of pirenzepine estimated for both gastric acid and salivary secretions were very similar, indicating little evidence of any selectivity of pirenzepine for gastric secretion.

References

ANGUS, J.A. & BLACK, J.W. (1979). Analysis of anomalous pK_B values for metiamide and atropine in the isolated stomach of the mouse. *Br. J. Pharmac.*, **67**, 59–65.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.* **14**, 48–58.

BLUM, A.L. & HAMMER, R. (1979) *Die Behandlung des Ulcus pepticum mit Pirenzepin.* Gräfelfing: Demeter-Verlag.

COLQUHOUN, D. (1971). *Lectures on Biostatistics.* Oxford: Clarendon Press.

DALY, M.J., HUMPHRAY, J.M. & STABLES, R. (1982). Effect of H_2-receptor antagonists and anticholinocepter drugs on gastric acid and salivary secretion induced by bethanechol in the anaesthetized dog. *Br. J. Pharmac.*, **76,** 361–365.

HAMMER, R., BERRIE, C.P., BIRDSALL, N.J.M., BURGEN, A.S.V. & HULME, E.C. (1980). Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature,* **283,** 90–92.

LEITOLD, M. & ENGELHORN, R. (1977). Pharmakologische Prüfung der magensekretionshemmenden Wirkung von 5,11-dihydro-11-[(4-methyl-piperazin-1-yl) acetyl]-6H-pyrido [2,3-b] [1,4] benzodiazepin-6-on-dihydrochlorid (LS 519). *Therapiewoche,* **27,** 1517–1531.

LEITOLD, M., ENGELHORN, R., BALLHAUSE, H., KUHN, F.J.A. ZIEGLER, H. Allgemeine pharmakodynamische Eigenschaften des Sekretions- und Ulkushemmers 5, 11-dihydro-11- [(4-methyl-piperazin-1-yl) acetyl]-6 H-pyrido [2,3-b] [1,4] benzodiazepin-6-on-dihydrochlorid (LS 519), *Therapiewoche,* **27,** 1551–1564.

MAIN, I.H.M. & PEARCE, J.B. (1981). pA_2 determination of muscarinic and H_2-receptor antagonists on gastric acid secretion *in vitro. Br. J. Pharmac.* **74,** 969–970P.

PARSONS, E., BUNCE, K.T., BLAKEMORE, C. & RASMUSSEN, C. (1979). Pharmacological studies on the gastric antisecretory agent, pirenzepine. In *Die Behandlung des Ulcus pepticum mit Pirenzepin* ed. Blum, A.L. & Hammer, R. pp. 26–33. Gräfelfing: Demeter-Verlag.

SZELENYI, I. (1981). An isolated mammaliam stomach preparation for studying the effect of substances on gastric acid secretion. *Arzn. Forsch. (Drug Res.),* **31,** 998–1000.

SZELENYI, I. & VERGIN, H. (1980). Cholinergic pathway of gastric acid secretion in the isolated whole stomach of the mouse. *Pharmacology,* **21,** 268–276.

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