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Comparative studies of the effects of some antimuscarinic agents on gastric damage and pupillary reflex in the rat

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The effects of some antimuscarinic compounds on oxotremorine-induced gastric damage, the pupil size and the pupillary light reflex have been studied in the rat. Unlike atropine, propantheline and methylscopolamine, pirenzepine is effective in preventing gastric erosions at doses much lower than those that affect pupillary reflex.

Introduction Pirenzepine is an antimuscarinic agent which has been shown to differentiate between different subtypes of muscarinic receptors (Hammer *et al.*, 1980). This concept of selectivity has been extended to the gastrointestinal tract since many studies have indicated that pirenzepine is effective in healing peptic ulcer and inhibiting acid secretion but has almost none of the typical side effects of the conventional antimuscarinic agents (Jaup, 1981). Recently, contradictory findings on this topic have been published. Some authors have shown that pirenzepine does not have antisecretory activity at doses lower than those inducing side effects (Main & Pearce, 1981; Szelenyi, 1982; Daley *et al.*, 1982), while other authors confirmed and supported the existence of selectivity for pirenzepine (Del Soldato *et al.*, 1982a; Perry & Heathcote, 1982; Hirschowitz *et al.*, 1983). In detail, according to Perry & Heathcote (1982) pirenzepine, unlike the conventional antimuscarinic drugs, is more effective in suppressing acid secretion than it is in increasing pupil diameter. In addition, the mydriatic response is not a specific antimuscarinic response (Bowman & Rand, 1980) and it is quite important to evaluate 'pure' antimuscarinic effects on the pupil, since this effect appears to be one of the most serious adverse reactions to antimuscarinic therapy (Weiner, 1980). Therefore, it seemed worthwile to evaluate further the effects on the pupillary reflex and to compare the effects of pirenzepine, atropine, methylscopolamine and propantheline on it and on gastric damage, both of which are specifically mediated by muscarinic receptor activation.

Methods Female albino rats, Sprague Dawley strain, 120–150 g, in groups of 10, were housed in plastic cages with wire bottoms to minimize coprophagy and fasted for 24 h before the experiments, with water *ad libitum*. Ten rats each were used for the control and drug-treated groups.

At least three doses of antimuscarinic compound were given to obtain dose-response curves. Atropine sulphate (BDH), methylscopolamine (BDH), propantheline methylbromide (Searle) and pirenzepine (Thomae) were freshly dissolved in 0.9% w/v NaCl solution (saline) and administered intravenously (i.v.). Doses are expressed as free bases. Oxotremorine and neostigmine (Sigma) were dissolved in 0.16 M phosphate buffer, pH 7.4, and the solutions were used for 4–5 days.

By use of a binocular microscope with a graduated scale in one eyepiece, pupil diameters were measured before and 15 min after the i.v. administration of the antimuscarinic agent, with or without a light stimulus. The light reflex was tested by lighting the right eye with a quartz-rod light for 15 s. The procedure for inducing gastric ulcer was as previously described (Del Soldato *et al.*, 1982a).

Oxotremorine was given i.v. at a dose of 700 $\mu g\,kg^{-1}$, 30 min after neostigmine (3 $mg\,kg^{-1}$, orally). Antimuscarinic agents were injected i.v. 15 min before oxotremorine. This time interval was selected since it was found in preliminary time-response studies to be the time of the peak effect for the anti-ulcer and the mydriatic activity of the compounds.

The effective $dose_{200}$ (ED_{200}) (the dose which doubled the diameter of the unstimulated pupil) and the ED_{50} (the dose which inhibited by 50% either the miotic response induced by light or the incidence of oxotremorine-induced lesions) were determined for antimuscarinic compounds by means of regression analysis, using standard procedures (Saunders & Fleming, 1971).

Results The effects of graded intravenous doses of pirenzepine, atropine, methylscopolamine and prop-

Table 1 Effect of some antimuscarinic agents on gastric lesions, pupil size and pupillary light reflex in rats

Compounds	$\mu g\,kg^{-1}$ for:			*Therapeutic index*	
	ED_{50} *gastric damage* (a)	ED_{200} *pupil diameter* (b)	ED_{50} *pupil reflex* (c)	b/a	c/a
Atropine	2.9 (1.6–4.3)	4 (2–7)	16 (15–17)	1.4	5.5
Propantheline	0.7 (0.3–1.6)	3 (2–4)	7 (5–8)	4.3	10
Methylscopolamine	0.4 (0.2–0.7)	1.8 (1.3–2.3)	3.6 (2.5–5.1)	4.5	9
Pirenzepine	19.0 (8.8–40.9)	408 (226–735)	2064 (1744–2442)	21.5	109

In parentheses: 95% fiducial limits.

antheline on oxotremorine-induced gastric damage and pupillary function and size are shown in Table 1. In order to evaluate the selectivity of the drugs for the stomach, the ED_{50} for inhibition of the pupillary light reflex or the ED_{200} for the increase in pupil diameter were expressed as ratios to the ED_{50} for inhibition of gastric damage. As can be seen, the ratios for pirenzepine are 15 and 20 times greater than those for atropine, 5 and 11 times those of propantheline and 5 and 12 times those of methylscopolamine, indicating that the first compound is more selective for the stomach than the other three.

Discusion The selective profile of antimuscarinic compounds is evaluated by analysis of the ratios of the therapeutic doses to the doses that induce side effects. Although in most papers about the selectivity of anti-ulcer compounds with antimuscarinic properties, the anti-secretory activity is used as the index for therapeutic effects, anti-ulcer activity should be better, since there is growing evidence that pirenzepine and antimuscarinics in general possess anti-ulcer properties, which may be partially independent of acid secretion inhibitory activity (Del Soldato *et al.*, 1982b; Rovati *et al.*, 1982). An ulcerogenic agent with specific parasympathomimetic properties (Cho *et al.*, 1962) was used for this study.

One of the side effects of most antimuscarinic drugs, that is the influence on the pupil, was studied because of the number of the possible consequences (such as impairment of near vision, risk of damage to the retina, increased intraocular pressure in people predisposed to narrow-angle glaucoma, etc.) of this group of drugs (Weiner, 1980). The mydriatic response is commonly used as the index of pupillary function in animal studies, but this measure is not a specific antimuscarinic response. In fact, antimuscarinic compounds are both mydriatic (i.e., they produce dilatation of the pupil through paralysis of constrictor pupillae) and cycloplegic (i.e., they cause paralysis of the ciliary body and accommodation, with a resultant dilatation of the pupil). They differ from other agents that cause pupillary dilatation through contraction of dilatator pupillae (such as adrenergic compounds) without loss of accommodation and the pupillary light reflex. Therefore, it is clear that the mydriatic response may be due to either a specific antimuscarinic interaction or to a non-specific effect, or both, and it is important to find a model based on pupillary antimuscarinic effects and predictive of impairment of accommodation or risk of glaucoma.

Since one of the most constant and significant clinical signs in glaucoma is full dilatation of the pupil with no response to light stimuli (Magrane, 1974), the evaluation of both pupil diameter and the light reflex might reliably test for specific antimuscarinic effects. Our present data show that, unlike atropine, propantheline or methylscopolamine, pirenzepine elicits anti-ulcer effects at doses significantly lower than those that induce mydriasis or cause the loss of the pupillary light reflex, giving a therapeutic dose range within which pirenzepine can exert its anti-ulcer activity without inducing any ocular side effect in rats.

Lastly, the clinical significance of these findings should be emphasized. Our data are in agreement with clinical reports (Jaup, 1981) that pirenzepine produced fewer ocular side effects than conventional antimuscarinics.

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