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OCCURRENCE OF H_1- AND H_2-HISTAMINE RECEPTORS IN THE GUINEA-PIG GALL BLADDER *in situ*

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1 Histamine has a dual action on the *in situ* gall bladder of the guinea-pig: a spasmogenic and a relaxant effect mediated through H_1- and H_2-receptor stimulation respectively.

2 The contracturant action, mimicked by 2-(2-aminoethyl) thiazole (a specific H_1-receptor agonist), is blocked by mepyramine and the relaxation, mimicked by dimaprit (a specific H_2-receptor agonist), is inhibited by cimetidine.

Introduction

Recently, two distinct types of histamine receptors have been described. The first type is blocked by the classical antihistamines such as mepyramine and is designated as type H_1 (Ash & Schild, 1966); the second is designated as type H_2 and is blocked by metiamide or cimetidine (Black, Duncan, Durant, Ganellin & Parsons, 1972).

The introduction of a specific H_1-receptor agonist, 2-(2-aminoethyl)thiazolene (Levi, Ganellin, Allen & Willms, 1975; Durant, Ganellin & Parsons, 1975), of H_2-specific agonists, N-methyl-2-(5-methyl-4-imidazolyl)ethylamine (Impicciatore Bertaccini, Mossini, Hansen & Grossman, 1977) and -[S-[3-(N, N dimethylamino)propyl]isothiourea] (Parsons, Owen, Ganellin & Durant, 1977) and on the other hand the discovery of a specific H_2-receptor blocking agent, cimetidine (Brimblecombe, Duncan, Durant, Emmet, Ganellin & Parsons, 1975), provided important tools in the identification and analysis of the histamine receptors.

The aim of this research was to investigate the actions of histamine on the guinea-pig gall bladder *in situ* in which it exerts a dose-dependent spasmogenic effect (Bertaccini, De Caro, Endean, Erspamer & Impicciatore, 1968) and to identify the receptors involved. Furthermore, some experiments have been performed with the synthetic octapeptide of cholecystokinin, in order to investigate whether this compound may in some way interact with histamine receptors.

Methods

The experiments were carried out on male guinea-pigs (250 to 450 g), anaesthetized with urethane (2 g/kg subcutaneously), prepared exactly as described by Ljungberg (1964). A midline incision about 2 cm long was made in the superior part of the abdominal wall. The gall bladder was gently separated from the adhering liver tissue, lifted cautiously into the wound and a thin thread sewn to its free pole.

The responses of the gall bladder to substances were recorded isometrically on a microdynamometer (Basile Ugo-Milano).

All the compounds were injected intravenously, via a thin polythene tube inserted into the jugular vein. Drugs used were: histamine hydrochloride (Merck); mepyramine(Fluka); cimetidine, -[S-[3-(N,N-dimethylamino)propyl]isothiourea] and 2-(2-aminoethyl)-thiazole (these compounds were kindly supplied by Smith Kline & French Laboratories); N-methyl-2-(5-methyl-4-imidazolyl)ethylamine hydrochloride (compound supplied by Institute of Pharmaceutical Chemistry, Parma, Italy); synthetic octapeptide of cholecystokinin (Squibb).

Results

Effects of histamine H_1- and H_2-receptor agonists

Histamine exerted a dose-dependent spasmogenic effect on the gall bladder of the anaesthetized guinea-pig. The threshold dose ranged between 0.25 and 0.5 μg/kg. Because of the well known side effects of histamine, the contractions elicited by its administration at doses of 8 to 10 μg/kg had to be considered as maximal responses.

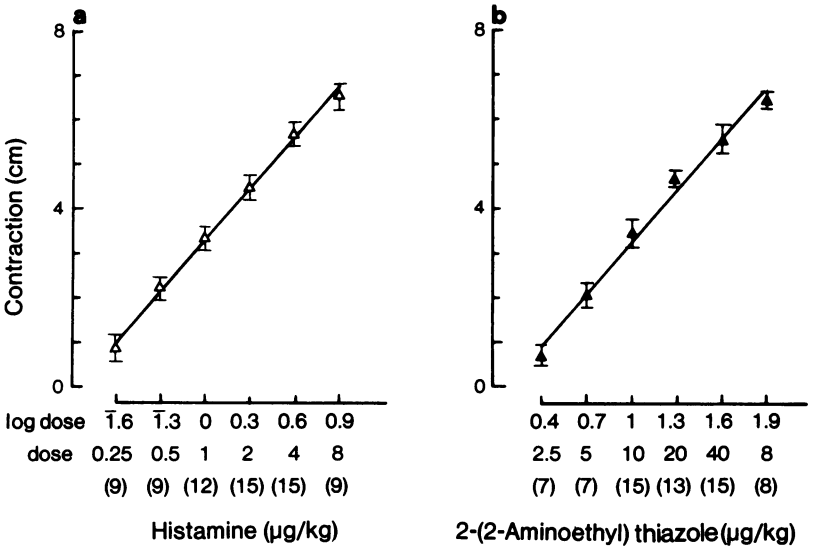

Figure 1 Contraction of the guinea-pig gall bladder *in vivo*. Single responses to histamine (△) and 2-(2-aminoethyl)thiazole (▲), injected intravenously. Ordinates: gall bladder contraction expressed in cm of tracing. Abscissae: doses of histamine (a) and 2-(2-aminoethyl)thiazole (b) (log scale) μg/kg. The lines are the calculated least squares regression lines r = 0.9972, $P < 0.001$, r = 0.9943, $P < 0.001$ respectively. The points are the mean results of between 7 and 15 separate experiments; vertical lines indicate s.e. means.

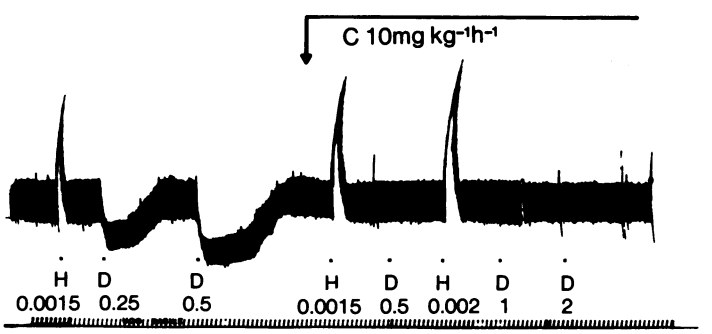

Figure 2 Guinea-pig gall bladder *in vivo*. Contraction and relaxation elicited by histamine (H) and dimaprit (D) respectively (mg/kg). Cimetidine (C) administered at a dose of 10 mg $kg^{-1}h^{-1}$ potentiated the contractile effect of histamine and completely blocked the relaxant effect of dimaprit even at high doses (1 to 2 mg/kg).

Similar results were obtained with 2-(2-aminoethyl)-thiazole, a specific H_1-receptor stimulant, although its threshold dose ranged between 2.5 to 5 μg/kg. Dose-response relationships for both compounds are shown in Figure 1.

The compound N-methyl-2-(5-methyl-4-imidazolyl) ethylamine given in doses of 1 to 2 mg/kg caused a very weak, short-lived contraction of the gall bladder followed by predominant relaxation.

Dimaprit, an H_2-receptor agonist, produced only a relaxation of the gall bladder musculature at all dose levels; the intravenous threshold dose was of the order of 150 to 250 μg/kg and a good dose-response relationship was always noted (Figure 2). When a maximal response had been obtained, increased doses produced more prolonged effects.

The synthetic octapeptide of cholecystokinin was the most powerful stimulant of the gall bladder, among the drugs examined, its threshold dose being 0.01 to 0.02 ng kg.

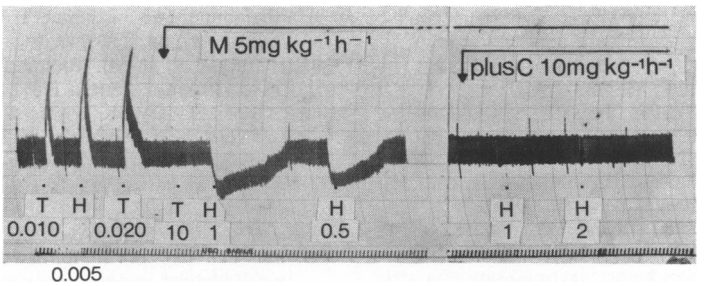

Figure 3 Guinea-pig gall bladder *in vivo*. Contractions of gall bladder elicited by histamine (H) and 2-(2-aminoethyl)thiazole (T) administered intravenously (mg/kg). Infusion of mepyramine (M) (5 mg $kg^{-1}h^{-1}$) completely blocked the spasmogenic effect of both compounds leaving a relaxant effect at higher doses of histamine. 2-(2-Aminoethyl)thiazole did not provoke any relaxation after mepyramine. A mixture of mepyramine (5 mg $kg^{-1}h^{-1}$) plus cimetidine (C) (10 mg $kg^{-1}h^{-1}$) completely blocked any effect of histamine.

Effects of histamine H_1- and H_2-receptor antagonists

The intravenous injection (500 μg/kg) or intravenous infusion (5 mg $kg^{-1}h^{-1}$) of mepyramine, a potent H_1-receptor antagonist, inhibited the gall bladder contraction induced by histamine and a (dose-dependent) relaxant effect of the amine was observed (Figure 3).

Similarly, mepyramine completely blocked the contracture produced by the H_1-specific agonist, 2-(2-aminoethyl)thiazole; in this case, however, no relaxant effect was observed, even administered at high dose levels (Figure 3).

The minor spasmogenic effect of N-methyl-2-(5-methyl-4-imidazolyl)ethylamine was inhibited by mepyramine, while the predominant relaxant effect was slightly potentiated.

Cimetidine, given as a single injection or by continuous infusion (5 to 10 mg $kg^{-1}h^{-1}$) potentiated the spasmogenic response to low doses of histamine and inhibited the relaxant effects elicited by N-methyl-2-(5-methyl-4-imidazolyl)ethylamine and dimaprit (Figure 2); a mixture of mepyramine plus cimetidine completely blocked the whole effect of histamine (Figure 3). Neither 2-(2-aminoethyl)thiazole nor dimaprit were modified by cimetidine and mepyramine respectively (at doses used in all other experiments). The spasmogenic action of the octapeptide of cholecystokinin, was not appreciably affected either by mepyramine or cimetidine at doses up to 10 and 20 mg/kg respectively.

Discussion

Results obtained in the present study, show that the gall bladder of the guinea-pig possesses both H_1- and H_2-receptors in accordance with the *in vitro* results obtained by Waldman, Schebalin, Zfass & Makhlouf (1976).

Histamine exerts a dual action; the predominant spasmogenic effect is probably mediated through excitation of H_1-receptors. In fact: (a) the effect was completely blocked by mepyramine, a specific antagonist for H_1-receptors; (b) the effect was potentiated by cimetidine, a specific antagonist for H_2-receptors; finally, (c) the effect was mimicked by 2-(2-aminoethyl)thiazole, a specific H_1-receptor stimulant.

Conversely, the relaxant action shown by histamine on the same musculature, after mepyramine pretreatment, is attributable to stimulation of H_2-receptors. In fact: (a) the effect was completely blocked by cimetidine, a specific H_2-receptor antagonist; (b) the relaxant effect was mimicked by N-methyl-2-(5-methyl-4-imidazolyl)ethylamine, which is known to be a prevalent H_2-agonist, and even more so by dimaprit, which is a pure H_2-agonist. As expected, cimetidine caused a complete blockade of the relaxant effects of both N-methyl-2-(5-methyl-4-imidazolyl)ethylamine and dimaprit.

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