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ACTION OF HISTAMINE RECEPTOR AGONISTS AND ANTAGONISTS ON THE RAT UTERUS

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1 Histamine and a series of compounds acting selectively on H_1- and H_2-receptors were tested on the isolated oestrous uterus of the rat.

2 Histamine had a dose-dependent inhibitory effect on the contractions elicited by acetylcholine. This action was unaffected by H_1-blockers but was competitively inhibited by H_2-blockers. The H_1-selective agonist, 2-(2-aminoethyl)thiazole was ineffective at doses 100 times greater than those of histamine. Conversely, all the H_2-agonists showed activity in the order of potency: *N'*-methylhistamine > histamine > *N'*-*N'*-dimethylhistamine > 5-methylhistamine > 5-methyl-*N'*-methylhistamine. Among the non-imidazole compounds, dimaprit had an activity identical to that of histamine, but all the dimaprit-like compounds showed negligible activity.

3 The data obtained suggest that in the rat uterus, (a) the activation of H_2-receptors is responsible for the inhibitory effect of histamine and its analogues; (b) the integrity of the histamine molecule seems to be less crucial than that of the dimaprit molecule for the maintenance of the H_2-activity, since changes in its structure modify but do not abolish the biological activity as they do in the case of dimaprit; (c) the order of activity of the various H_2-receptor agonists is different from that observed in other tissues.

Introduction

The mechanism of the relaxant effect of histamine on the rat uterus is controversial. Some investigators have suggested that histamine acts by direct (Jensen & Vennerod, 1961) or indirect (Tozzi, 1973) stimulation of adrenoceptors, whereas others (Black, Duncan, Durant, Ganellin & Parsons, 1972) have suggested that the histamine-induced relaxation is associated with activation of the H_2-receptors. This hypothesis was supported by the demonstration of the competitive inhibition of the histamine effect, elicited by the first of the classical H_2-antagonists, burimamide, which, however failed to modify isoprenaline-induced relaxation. No extensive study of the interaction of histamine and the H_2-receptors of the rat uterus has appeared in the recent literature.

Since we are making an extensive study of the distribution of the two types of histamine receptors in various tissues of different animal species, we decided to investigate thoroughly the effects of histamine and of different compounds acting on the H_1- and H_2-receptors as selective stimulants or inhibitors, on the rat uterus and the results are described in this paper.

Methods

Experimental animals and procedure

Ovariectomized adult rats weighing approximately 140 g were used. They were injected once or twice with 50 to 100 μg oestradiol dipropionate 5 to 7 days before they were used. The rats were killed by decapitation and uteri were rapidly removed and suspended in a 10 ml bath of de Jalon solution of the following composition (g/l): NaCl 9, KCl 0.42, $CaCl_2$ 0.06, glucose 0.5 and $NaHCO_3$ 0.5, kept at 32°C and bubbled with air. Contractions of the uterus recorded by means of an isotonic lever on a kymograph were obtained by administering acetylcholine (5×10^{-6} M) at regular intervals of 1 min. Only the height of the contractions was considered. The inhibitory effect of histamine and related compounds was calculated as the percentage reduction of the contractions elicited by a dose of acetylcholine given 2 min after histamine or its analogues. In a second series of experiments some antagonists of the H_1- or H_2-type were administered immediately before the agonists; in this case both the degree and the duration of the inhibition of the effect of the agonist were evaluated.

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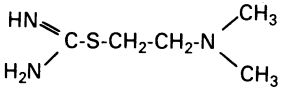
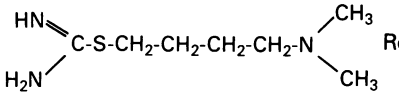
Drugs

The following drugs were used besides histamine (Fluka): (1) H_2-agonists: *N'*-*N'*-dimethylhistamine (DMH), *N'*-methylhistamine (MMH), 5-methylhistamine (5MH), this compound can also be called 4-methylhistamine according to the numbering of the side chain of histamine which can be considered either as a 2-(4-imidazolyl)ethylamine or as a 2-(5-imidazolyl)ethylamine, 5-methyl-*N'*-methylhistamine (5MNMH, this compound can also be called 4-methyl-*N'*-methylhistamine), 2-(5-methyl-4-imidazolyl)-1-methyl-ethylamine (compound Hi56), dimaprit (SK & F), compounds Ros 2, Ros 4, Ros 5; (2) H_1-agonist: 2-(2-aminoethyl)thiazole (SK & F); (3) H_2-antagonists: metiamide and cimetidine (SK & F); (4) H_1-antagonists: pyrilamine and chlorpheniramine (Fluka). All the compounds used in the present experiments, unless otherwise specified, were synthesized by one of us (T.V.). Molarity of the compounds used in the present experiments, refers to the active components.

The structures of the less familiar compounds are represented below.



Ros 2



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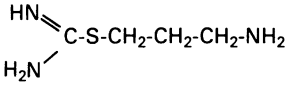
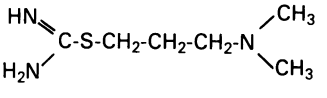
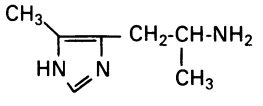


Ros 5



Dimaprit



Hi 56

Results

Agonists

Histamine exerted the expected inhibitory effect on uterine contraction stimulated by acetylcholine; the threshold dose was approximately 2×10^{-6} M and there was a good dose–response relationship up to the maximal dose (5×10^{-4} M), which completely blocked the effect of acetylcholine.

In the group of imidazole derivatives, all the compounds examined showed the same behaviour as the parent substance, as is demonstrated by the dose–response curves shown in Figure 1. However, except for the *N'*-methyl derivative the potencies of all the other compounds were less than that of histamine. The pD_2 values for the different substances are shown in Table 1.

We did not notice with the rat uterus the long-lasting duration of the inhibitory effect of the methyl derivatives which was observed under other experimental conditions (Bertaccini & Impicciatore, 1974; Impicciatore, Bertaccini, Chiavarini, Molina, Vitali & Bordi, 1978a). Among other non-imidazole H_2-receptor agonists, dimaprit, one of the most selective H_2-agonists (Parsons, Owen, Durant & Ganellin, 1977) had a striking relaxant effect, comparable to that elicited by histamine. The dose–response curve overlapped that of histamine but its derivatives were virtually inactive, being less than 1/100 as potent as dimaprit.

The H_1-receptor selective agonist, 2-(2-aminoethyl)-thiazole (Durant, Ganellin & Parsons, 1975) was, under our experimental conditions, absolutely ineffective in doses up to 100 times greater than that of histamine.

Antagonists

The H_1-antagonists failed to modify the effects of histamine and of the other agonists at all doses tested (up to 4×10^{-6} M). Conversely, both metiamide and cimetidine caused a remarkable inhibition of the effect of histamine. Most of the experiments were performed with metiamide and are summarized in Figure 2. Cimetidine, tested only in a few cases ($n = 12$) behaved approximately as metiamide. The pA_2 of metiamide under our experimental conditions was 6.11, that of cimetidine 6.05. An example of the inhibitory effect of cimetidine against the effect of histamine and that of dimaprit is shown in Figure 3.

Discussion

Under our experimental conditions, the well known relaxant effect of histamine on the rat uterus was shown to be absolutely independent of the excitation of the H_1-receptors. The H_1-selective agonist, 2-(2-aminoethyl)thiazole, failed to mimic the inhibitory effect of histamine even administered in concentrations more than 100 times that of histamine. In addition, the H_1-receptor blockers, pyrilamine and

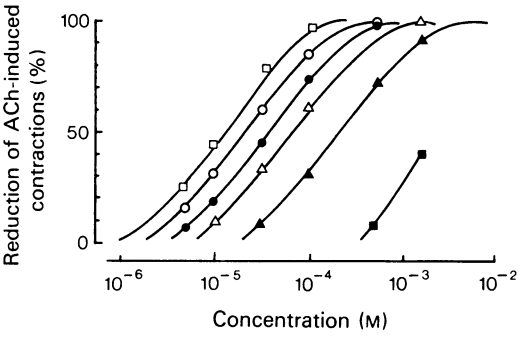


Figure 1 Dose–response curves to various imidazole compounds of the rat uterus. On the ordinate scale, % reduction of the contractions induced by acetylcholine (5×10^{-6} M) are shown; on the abscissa scale molar concentrations (M) of the different compounds: (□) *N'*-methylhistamine; (○) histamine; (●) *N'*-*N'*-dimethylhistamine; (△) 5-methylhistamine; (▲) 5-methyl-*N'*-methylhistamine; (■) compound Hi56. Each value refers to the mean of the values obtained from 10 to 20 experiments.

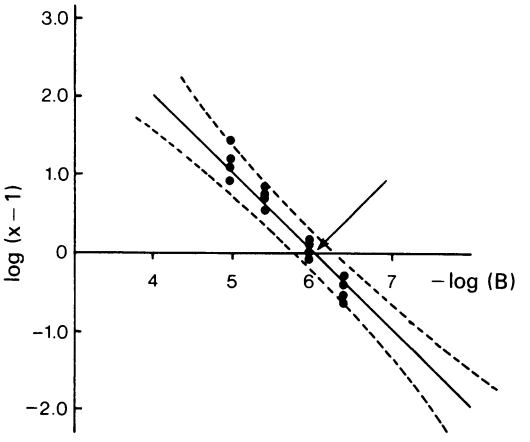


Figure 2 Plot of equation (1). Histamine as agonist and metiamide as antagonist ($-n = 1.03 \pm 0.14$). Arrow indicates the pA_2 value (6.11). Dashed lines indicate 95% confidence limits.

chloropheniramine, failed to modify the action of histamine. In this respect the rat uterus appeared to be completely different from the human and guinea-pig uterus, in which the action of histamine is stimulation and not inhibition and is apparently associated with an interaction with H_1-receptors (Molina, Zappia & Zinelli, 1976; Molina & Zappia, unpublished).

Results obtained with metiamide and cimetidine suggested that the two compounds behaved as simple competitive antagonists, in fact plotting the data according to equation (1) (Schild, 1949; Arunlakshana & Schild, 1959)

$$\log(x - 1) = \log K_2 - npAx \qquad (1)$$

the results can be fitted by a straight line with a slope of $-n = 1.03$ and 0.99 for metiamide and cimetidine respectively. This is in accordance with the data of Black *et al.* (1972) and of Mitznegg, Schubert & Fuchs (1975).

As far as the various H_2-agonists were concerned, the situation was different for the imidazole and the non-imidazole compounds.

Imidazole compounds

In this group all the compounds were active though to various degrees. *N'*-methylhistamine was actually more potent than histamine itself and the ratios of potency of most of the compounds examined were the same as those found by Holton & Spencer (1976) for the guinea-pig isolated stomach which is another suitable tissue for the study of H_2-receptors. 5-Methyl-*N'*-methylhistamine which was not investigated by Holton & Spencer (1976), was found by Impicciatore, Morini & Bertaccini (1978b) to be approximately as active as histamine in the guinea-pig

Table 1 pD_2 values of the different imidazole compounds on the isolated uterus of the rat

Compound	pD_2	*s.e. mean*	n
N'-methylhistamine	4.91	±0.30	42
Histamine	4.68	±0.27	180
N'-*N'*-dimethylhistamine	4.50	±0.30	75
5-Methylhistamine	4.17	±0.26	54
5-Methyl-*N'*-methylhistamine	3.66	±0.28	12
Comp. Hi 56*	2.62	—	7

n = number of experiments

*This compound could not be tested up to the maximal dose because there was not enough of it; the pD_2 value represents the expected theoretical value.

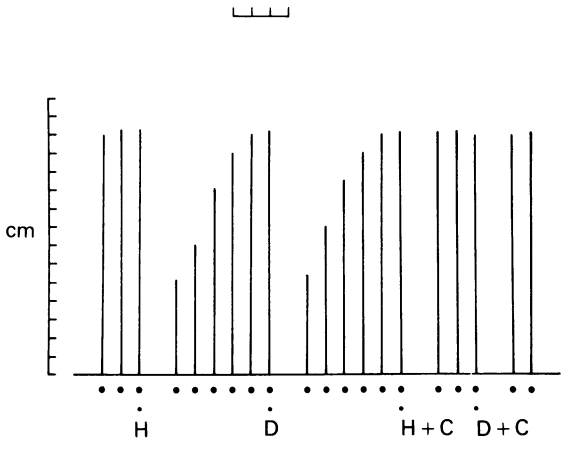


Figure 3 Oestrous uterus of rat. Effect of cimetidine (C) on the inhibitory effect of histamine (H) and dimaprit (D) against the contractions induced by acetylcholine (5×10^{-6} M) given at each dot. Doses of cimetidine, histamine and dimaprit (all 3×10^{-5} M). Vertical scale represents height of contractions in cm. Time = 1 min.

isolated stomach, whereas in the present experiments it retained only 1/10 of the activity of histamine. The same compound was found to be twice as active as histamine in stimulating gastric secretion in conscious cats (Bertaccini, Impicciatore & Vitali, 1976). Other results obtained *in vivo* with the *N'*-methylated compounds showed that not only *N'*-methyl- but also *N'*-*N'*-dimethylhistamine was more (about twice) potent than the parent substance (Code, Maslinski, Mossini & Navert, 1971), but the same was also true for 5-methylhistamine (Bertaccini & Impicciatore, 1974). Obviously the discrepancies between *in vitro* and *in vivo* experiments may be associated with haemodynamic or kinetic factors and cannot be used to argue that histamine H_2-receptors are different in the various tissues. The activity of the compound labelled Hi 56, although approximately 1/100 that of histamine, is rather interesting since this substance has been shown (Bertaccini, 1978; Impicciatore *et al.*, 1978a) to be virtually ineffective on H_1-receptors, with a ratio of activity H_2-/H_1-receptors of more than 500. This ratio fell to 2.5 in the analogue which lacked the CH_3 group in the imidazole ring [2-(4-imidazolyl)-1-methylethylamine], as reported by Durant, Emmett, Ganellin, Roe & Slater (1976) and this once again emphasizes the importance of the methyl group in position 5 of the imidazole nucleus in enhancing the activity on the H_2-receptors.

Non-imidazole group

In this group, the situation was completely different. While the prototype, dimaprit, had an effect equivalent to that of histamine, all its derivatives showed very feeble, if any, activity. In any case, their activity was no more than 1/100 that of dimaprit. Therefore, looking at the structure–activity relationship in the two groups of substances examined, it is possible to state that changes in the histamine molecule may cause not only a more or less significant decrease in the activity but can actually potentiate histamine-like action. In contrast the integrity of the dimaprit molecule is apparently crucial for the maintenance of its biological activity and even small changes produce a striking decrease in activity. Of course the possibility that the discovery of new dimaprit-like molecules may invalidate this assumption, cannot be excluded. The lack of activity of the dimaprit analogues was also observed in atria (Coruzzi, Plazzi & Bertaccini, 1978), papillary muscle (Bertaccini, Coruzzi & Vitali, 1978), isolated stomach (Impicciatore, Morini, Bordi & Bertaccini, 1978c) and gall bladder (Impicciatore *et al.*, unpublished) of the guinea-pig. For comparison, dimaprit was approximately as active as histamine in the present experiments, but was found to be only 1/5 as effective as histamine in the electrically stimulated rat uterus and 4 to 5 times more effective than histamine in stimulating gastric secretion from anaesthetized cats (Parsons *et al.*, 1977). Quite recently, it was observed that this compound had approximately 70% of the activity of histamine with a similar maximal response in the guinea-pig auricle (Parsons *et al.*, 1977; Coruzzi *et al.*, 1978), but it had only 10% of the activity of histamine on the contraction of the papillary muscle of the guinea-pig (Bertaccini *et al.*, 1978). All these observations suggest the possibility that the distribution of H_2-receptors in the different tissues may be heterogeneous from a quantitative and/or qualitative point of view as proposed by Angus, Black & Stone (1978). This situation finds its counterpart in the field of the sympathetic β-adrenoceptors which were found to be different in the heart and in the bronchial muscle and in the uterus, and for which a subclassification (β_1- and β_2-receptors) was suggested (Lands, Arnold, McAuliff, Luduena & Brown, 1968).

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