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Histamine receptors in the guinea-pig duodenum

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Guinea-pig duodenum contracted by histamine or by acetylcholine was relaxed dose-dependently by a series of H_2-receptor selective agonists namely dimaprit, impromidine, clonidine and tolazoline. This relaxation was not neurally mediated since it was not modified by tetrodotoxin nor was it exerted through sympathetic receptors because it was not modified by pretreatment with propranolol or phentolamine. Apparently it was connected with the H_2-receptor stimulation more than to peculiarities of the single compounds. However a series of H_2-blockers (metiamide, cimetidine, ranitidine or oxmetidine) were unable to counteract the effect of the H_2-agonists or the relaxant effect of histamine in the presence of chlorpheniramine. This peculiar situation seems to indicate the existence of anomalous H_2-receptors, susceptible to the action of the agonists but not to that of the antagonists.

Bareicha & Rocha e Silva (1975) reported that burimamide potentiated histamine-induced contractions of guinea-pig duodenum. They interpreted this as being due to the abolition of the relaxing effect, mediated through stimulation of H_2-receptors, that is usually masked by the overwhelming presence of the H_1-receptors. In preliminary experiments on guinea-pig duodenum and ileum (Bertaccini et al 1979a b; Bertaccini & Zappia, unpublished) we were unable to observe any potentiation of the effect of histamine by using other, more recent H_2-receptor blockers, like cimetidine and ranitidine. On the other hand we observed that dimaprit, an H_2-receptor agonist (Parsons et al 1977), caused a remarkable relaxant effect on the histamine-induced contraction thus supporting the existence of H_2-receptors the stimulation of which cause relaxation of the duodenum. The availability of new, potent and selective agonists and antagonists of the H_2-receptors prompted us to investigate more thoroughly the occurrence and the possible role of histamine receptors in the guinea-pig duodenum. The inhibitory effect of the H_2-receptor agonists was tested also on the submaximal contraction elicited by acetylcholine.

METHODS

Guinea-pigs of either sex, ca 300 g, were killed and the duodenum removed, placed in a 10 ml bath at 37 °C bubbled with 95% oxygen and 5% CO_2 mixture in a Krebs–Henseleit solution. Contractions were recorded by an isometric transducer and a microdynamometer (Basile, Comerio). The preload of the muscle was 0·5 g tension; the resting tension was 0·25 g.

Contracting drugs (histamine or acetylcholine) were adminstered at intervals of 10 min. Submaximal doses (about 0·1 μg ml^{-1}) capable of evoking 90% of the maximum response (1·2 g tension) were used for both compounds. The H_2-stimulants were given at the time of maximum contraction. When the inhibitors were given they were allowed to act for 15 min. In a few experiments instead of using exogenous H_2-agonists, histamine in the presence of chlorpheniramine (30 ng ml^{-1}) a well known H_1-receptor antagonist was employed: in these experiments contracting drugs were represented by acetylcholine (usual dose) or KCl (0·1 mg ml^{-1}).

Drugs used were: histamine, tolazoline, phentolamine, acetylcholine and chlorpheniramine (Fluka); cimetidine, dimaprit, impromidine and oxmetidine (compound marked SKF 92994) (Smith Kline and French); ranitidine (Glaxo); clonidine (Boehringer Ingelheim); propranolol (ICI); tetrodotoxin (Sankyo).

RESULTS

Histamine and acetylcholine exerted their usual, stimulatory effect on the preparation starting from threshold doses of 0·5–3 ng ml^{-1} and reaching the maximum response with approximately 100–150 ng ml^{-1}. When the H_2-agonists were given they exerted a prompt dose-dependent relaxation of the duodenum (Tables 1, 2). In addition to dimaprit and impromidine, (Durant et al 1978) the most selective H_2-agonists so far known, clonidine and tolazoline were also used since these sympatholytic agents were found to possess a noticeable stimulatory effect on

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Table 1. Relaxant effect of different H_2-receptor agonists on the duodenal contraction induced by histamine. Numbers (mean ± standard error) refer to the % inhibition of a submaximal duodenal contraction, taken as 100.

Compounds	Doses of the H_2-receptor agonists in $\mu g\ ml^{-1}$ 0·3	1	3	10	30	100
Dimaprit	—	—	—	19·4 ± 3·9	47·7 ± 8·8	83·8 ± 9·0
Impromidine	6·7 ± 3·3	42·5 ± 14·4	77·5 ± 10·3	—	—	—
Clonidine	0	3·9 ± 2·0	9·7 ± 2·7	28·1 ± 6·9	54·4 ± 7·6	91·5 ± 3·2
Tolazoline	0	11·4 ± 4·1	32·2 ± 10·4	58·4 ± 11·2	81·5 ± 9·1	—

Table 2. Relaxant effect of different H_2-receptor agonists on the duodenal contraction induced by acetylcholine. Numbers (mean ± standard error) refer to the % inhibition of a submaximal duodenal contraction, taken as 100.

Compounds	Doses of the H_2-receptor agonists in $\mu g\ ml^{-1}$ 1	3	10	30	100
Dimaprit	—	0	1·5 ± 1·0	20·2 ± 5·4	55·9 ± 6·3
Impromidine	—	0	1·3 ± 1·3	12·5 ± 8·0	79·2 ± 5·5
Clonidine	6·8 ± 1·2	13·9 ± 3·0	36·3 ± 5·6	65·0 ± 5·1	87·4 ± 5·4
Tolazoline	—	—	0	14·9 ± 11·3	57·8 ± 13·9

the H_2-receptors (Karppanen & Westermann 1973; Yellin et al 1975; Anttila & Westermann 1976). Moreover histamine plus chlorpheniramine was tested during the contractions elicited either by acetylcholine or KCl. In both cases histamine provoked a dose-dependent relaxation from 100 to 1000 ng ml^{-1}. An example of the relaxant effect of clonidine in comparison with that of dimaprit, is given in Fig. 1. It is evident from the Tables that

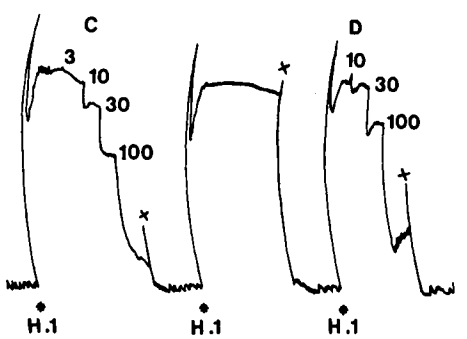

FIG. 1. Guinea-pig duodenum contracted by histamine (H). Relaxant effect elicited by clonidine (C) and dimaprit (D). Doses are in $\mu g\ ml^{-1}$. Time: 4 mm = 1 min. X = wash out.

impromidine was much more effective on histamine than on acetylcholine-induced contractions but this could be related to the well-known anti H_1-receptor activity of impromidine observed by Durant et al (1978) (PA_2 value on the guinea-pig ileum = 5·47) and by ourselves (Bertaccini & Zappia, unpublished; pA_2 value on the guinea-pig ileum = 5·40). Also, the other compounds were apparently more active on histamine—than on acetylcholine-induced contractions but differences were much less remarkable.

No antagonism of H_1-receptors could be demonstrated with tolazoline (up to 30 $\mu g\ ml^{-1}$). A series of compounds was given before the H_2-agonists to exclude interference from other mechanisms of action: tetrodotoxin (1 $\mu g\ ml^{-1}$), propranolol (0·3 $\mu g\ ml^{-1}$) and phentolamine (0·3 $\mu g\ ml^{-1}$). These drugs were ineffective against the four H_2-agonists.

Finally to test the hypothesis of an action mediated through stimulation of the classical H_2-receptors, a series of H_2-blockers was used. These were metiamide and cimetidine (up to 30 $\mu g\ kg^{-1}$) and the new potent drugs ranitidine (Bradshaw et al 1979; Woodings et al 1980) and oxmetidine (Blakemore et al 1980) (10 $\mu g\ ml^{-1}$). None of these agents was able to modify the relaxant effect of the H_2-agonist. The lack of the effect of ranitidine on the action of impromidine and dimaprit is shown in Fig. 2.

DISCUSSION

Our experiments showed that four H_2-receptor agonists namely dimaprit and impromidine (drugs known to be highly selective for the histamine receptors), clonidine and tolazoline (sympatholytic agents but also endowed with a stimulant effect on the H_2-receptors) were able to cause dose-dependent relaxation of histamine—or acetylcholine-induced contractions of the guinea-pig duodenum. Histamine in the presence of chlorpheniramine showed the same dose-dependent relaxation though very high

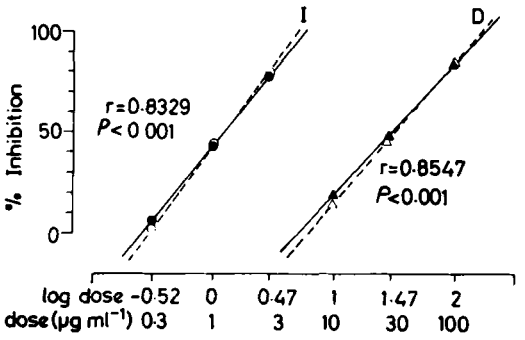

FIG. 2. Inhibitory effect (in %) of impromidine (I) and dimaprit (D) on the contraction induced by histamine on the guinea-pig duodenum. Continuous lines before ranitidine; interrupted lines after ranitidine (10 μg ml^{-1}). Lines are the least square regression lines.

doses were needed. This situation is similar to that observed in our laboratory (Impicciatore 1978; Bertaccini et al 1980) on the guinea-pig gall bladder. In this tissue a predominant spasmogenic effect was observed after administration of histamine or H_1-agonists. A relaxant effect was seen only with high doses of dimaprit or with high doses of histamine plus mepyramine. However, in the gall bladder the relaxant effect was blocked by cimetidine thus indicating an interference with the 'classical' H_2-receptors. The relaxant effect on the duodenum was not neurally mediated since it was not prevented by tetrodotoxin; moreover it was not mediated through stimulation of sympathetic α- or β-receptors since it was not blocked by phentolamine or propranolol.

All the four compounds though of different structure exerted the same qualitative effect with only quantitative differences; this suggested that the relaxant effect was not connected with a specific molecule but was rather a common property of these compounds i.e. H_2-receptor stimulation. On the other hand a series of H_2-antagonists (metiamide, cimetidine, ranitidine and oxmetidine) failed to inhibit the effect of the H_2-agonists. The explanation for this phenomenon is not entirely clear: a possible suggestion could be represented by the presence in the guinea-pig duodenum of H_2-receptors that are susceptible to the action of the agonists but not to that of antagonists. This situation is not unique and similar examples have been observed: relaxation of cat bronchi and trachea induced by 4-methylhistamine (a selective H_2-stimulant) was not modified by burimamide or metiamide (Chand & Eyre 1977), relaxation of ferret bronchi and trachea induced by 4-methylhistamine was not modified by burimamide, metiamide or cimetidine (Chand & Eyre 1978), reduction in noradrenaline content in the rat hypothalamus induced by histamine or 4-methylhistamine was not modified by metiamide or cimetidine (Nowak et al 1980).

The opposite situation has also been reported, i.e. effects of histamine that were presumably elicited through excitation of H_2 receptors (since they were blocked by H_2-antagonists) but which were not mimicked by H_2-agonists. This was the case for the inhibition of tetanic spasm by field stimulation induced by histamine but not mimicked by 4-methylhistamine (Ambache et al 1973) and for the extravasation of albumin and oedema induced by histamine (and blocked by metiamide) in the cat skeletal muscle not mimicked by dimaprit (Flynn & Owen 1979). It is probably premature to speak about sub-types of H_2-receptors and it is possible that artifacts due to the technique employed or differences in the tissues rather than in the receptors are responsible for the findings reported, as it was pointed out by Angus & Black (1980). However, the possibility of anomalous histamine-receptors different from the 'classical' H_2-receptors cannot be excluded on the basis of our experiments.

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