Br. J. Pharmac (1983), **78**, 011–016

Evidence against the temperature-dependent interconversion of histamine H_1- and H_2-receptors in the guinea-pig ileum

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1 The possible temperature-dependent interconversion of histamine H_1- and H_2-receptors in the guinea-pig ileum suggested from previous studies was re-investigated by use of new and selective H_2-receptor agonists and antagonists.

2 Chlorpheniramine, an H_1-blocker, caused a rightward shift of the cumulative histamine dose-response curve at both 37°C and 12°C. Conversely cimetidine and tiotidine, two H_2-receptor blockers, were ineffective at both temperatures. Metiamide behaved as a non competitive antagonist at 12°C but only in very high concentrations.

3 Dimaprit and impromidine, two selective H_2-receptor agonists, were inactive at both 37°C and 12°C when given alone, whereas at both temperatures they elicited the already described relaxation of the contractions induced by histamine.

4 Similar results were obtained on the guinea-pig whole ileum and on the longitudinal muscle strip: this indicates a lack of interference of the circular smooth muscle.

5 Our results allow us to conclude that no temperature-dependent interconversion of histamine H_1- and H_2-receptors occurs in the guinea-pig ileum.

Introduction

In 1974, Kenakin Krueger & Cook reported an interesting and quite peculiar phenomenon concerning histamine receptors. They observed in the isolated longitudinal strip of the guinea-pig ileum that, by changing the bath temperature from 37°C to 12°C, the histamine receptors responsible for the spasmogenic effect of the amine changed from the H_1- to the H_2-type. The dose-response curve to histamine obtained at 37°C was shifted to the right by tripelennamine; at 12°C and in the continued presence of tripelennamine some reversal of blockade occurred and this could be inhibited again with metiamide. This classical H_2-receptor antagonist was able to shift to the right the dose-response curve to histamine thus suggesting an interconversion of H_1- into H_2-receptors at the low temperature. The phenomenon of interconversion of receptors is well known and it was described by other authors for other kinds of receptors especially the adrenoceptors. Leaving aside the phenomenon of interconversion connected with different situations such as changes of thyroid hormone secretion, (Kunos, 1977; MacLeod & McNeill, 1980), the influence of adrenalectomy (Berthelsen & Pettinger, 1977) etc., the hypothesis of the temperature-dependent interconversion of receptors is a matter of remarkable controversy. Some authors reported that a reduction in temperature, converted β-into α-adrenoceptors in the frog heart (Buckley & Jordan 1970; Kunos & Nickerson, 1976) while other reports were completely at variance (Benfey, 1977; Bennet & Kemp, 1978). As for the histamine receptors, the experiments by Kenakin *et al.* (1974) were not convincing: the authors used only histamine and one H_2-antagonist (metiamide) and they did not pay any attention to the possible effects of H_2 selective agonists. The recent availability of new very potent and selective H_2- and H_1-agonists and antagonists and our specific interest in the field of histamine receptors because of the possible heterogeneity in this population (Bertaccini, 1981; Bertaccini & Coruzzi, 1981) prompted us to re-investigate the problem of the temperature-dependent interconversion. Our aim was to throw new light on this interesting experimental situation.

0007-1188/83/010011-06-$01.00

Methods

Guinea-pigs of either sex weighing approximately 300 g were used. The whole ileum, or the Auerbach plexus longitudinal muscle strip preparation according to Paton & Vizi (1969) were removed from freshly killed animals and suspended in an organ bath of 10 ml capacity in an oxygenated Krebs solution having the following composition (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose, 11.5. The initial tension of the transducer was 0.25 g: isometric contractions were recorded by a direct writing kymograph (Basile, Comerio). At a temperature of 37°C the complete dose-response curves to histamine or to 2-aminoethylthiazole were constructed: they were then repeated in the presence of H_1 or H_2-receptor antagonists. Afterwards the bath temperature was lowered (in approximately 2 h) to 12°C by means of cryothermostat Julabo Paratherm, and the same series of experiments was repeated.

Drugs

Compounds employed were: histamine and (+)-chlorpheniramine maleate purchased from Fluka; 2-aminoethylthiazole, metiamide, cimetidine, dimaprit and impromidine were generous gifts from Smith Kline and French Research Ltd., (Welwyn, England); tiotidine was a gift from I.C.I.

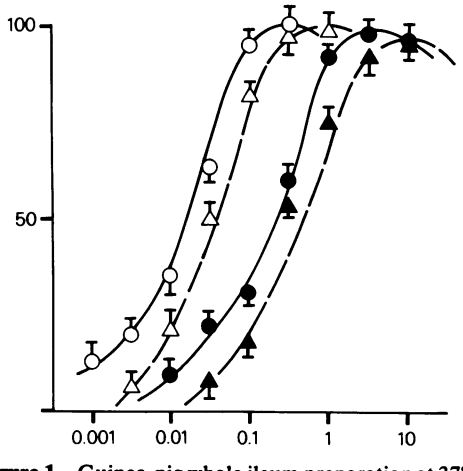

Figure 1 Guinea-pig whole ileum preparation at 37°C. Dose-response curves to histamine (○), 2-aminoethyliazole (△), histamine plus chlorpheniramine (3 ng/ml) (●), and 2-aminoethylthiazole plus chlorpheniramine (3 ng/ml) (▲). On the ordinate scale percentage of the maximum response; on the abscissa scale, doses of the agonists in μg/ml. Each value represents the mean of the value obtained from 5 to 20 experiments. Vertical bars are standard errors.

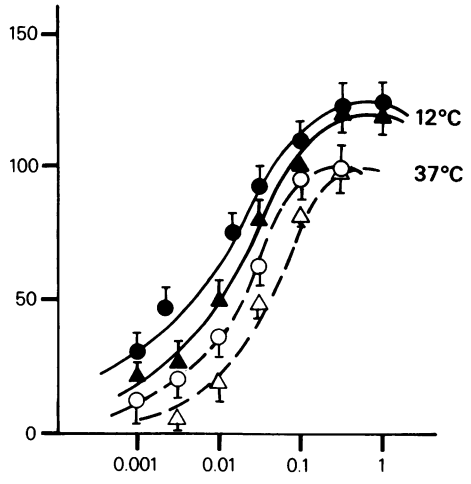

Figure 2 Guinea-pig whole ileum preparation. Dose-response curves to histamine (○, ●) and to 2-aminoethylthiazole (△, ▲) at 37°C and at 12°C respectively. On the ordinate scale, percentage of the maximum response obtained at 37°C; on the abscissa scale, doses of the agonists in μg/ml. Each value represents the mean of the values obtained from 5 to 10 experiments. Vertical bars are standard errors.

Results

Results obtained with histamine and 2-aminoethylthiazole at 37°C in the whole ileum were as expected: both compounds showed the classical dose-response curve which in both instances was

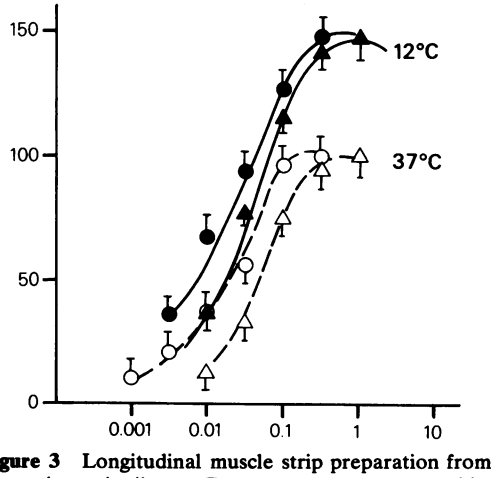

Figure 3 Longitudinal muscle strip preparation from the guinea-pig ileum. Dose-response curves to histamine (○, ●) and to 2-aminoethylthiazole (△, ▲) at 37°C and at 12°C respectively. On the ordinate scale, percentage of the maximum response obtained at 37°C; on the abscissa scale, doses of the agonists in μg/ml. Each value represents the mean of the values obtained from 4 to 8 experiments. Vertical bars are standard errors.

shifted to the right by pretreatment with chlorpheniramine (3 ng/ml) Figure 1. Dimaprit and impromidine were devoid of stimulatory effects up to concentrations of 30 μg/ml. Results obtained in the longitudinal muscle strip not shown in the figure overlapped those of the whole ileum. Cimetidine and tiotidine were unable to modify the dose-response curve to histamine up to concentrations of 30 μg/ml. The dose-response curves to histamine and to 2-aminoethylthiazole on both the whole ileum and the longitudinal muscle strip at 37°C and at 12°C are shown in Figure 2 and 3 respectively. It may be seen from these figures that in both preparations and especially in the latter, the maximum response to both agonists was consistently and significantly ($P<0.05$) higher at the low temperature. Results obtained in the whole ileum with histamine, 2-aminoethyl thiazole, alone and in the presence of the H_1-receptor antagonist chlorpheniramine and with the H_2-agonist dimaprit at 12°C are shown in Figure 4. Impromidine, not shown in the figure, behaved in the same way as dimaprit. It is evident that the dose-response curves to histamine and to 2-amino-ethyl thiazole were shifted to the right by chlorpheniramine indicating the competive antagonism of this compound and therefore pointing out that the two agonists contracted the guinea-pig ileum through activation of the classical H_1-receptors. pA_2 values based on the dose-ratios found with two concentrations of the antagonist (1 and 3 μg/ml) were 9.55 against histamine and 9.56 against 2-aminoethylthiazole in accordance with previous data in the literature (Van den Brink & Lien, 1977). We could not test higher concentrations of antagonist as this would have required an extremely high concentration of the agonist (2-aminoethylthiazole) which was not available. Therefore the Schild plot analysis could not be performed and the competitive antagonism could not be established with certainty. It is also clear from the figure that dimaprit was without any spasmogenic activity, a further confirmation of the absence of H_2-receptors endowed with a stimulatory effect. Very high doses of dimaprit (100 μg/ml) caused a relaxation of the contraction induced by

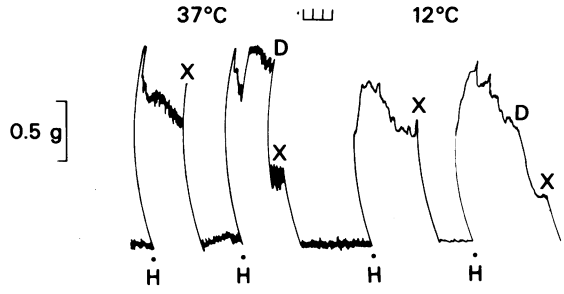

Figure 5 Guinea-pig whole ileum preparation at 37°C and at 12°C. H = histamine 100 ng/ml at 37°C and 30 ng/ml at 12°C. D = dimaprit 100 μg/ml at both temperatures. On the left, tension of the transducer in g. At (x), washing out of the preparation. Time in min.

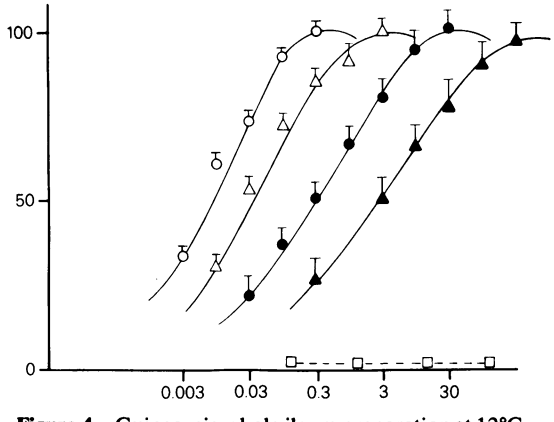

Figure 4 Guinea-pig whole ileum preparation at 12°C. Dose-response curves to histamine (○); 2-aminoethylthiazole (△); histamine plus chlorpheniramine (3 ng/ml) (●); 2-aminoethylthiazole plus chlorpheniramine (3 ng/ml) (▲) and dimaprit (□). On the ordinate scale, percentage of the maximum response; on the abscissa scale, doses of the agonists in μg/ml. Each value refers to the mean of the values obtained from 4 to 12 experiments. Vertical bars are standard errors.

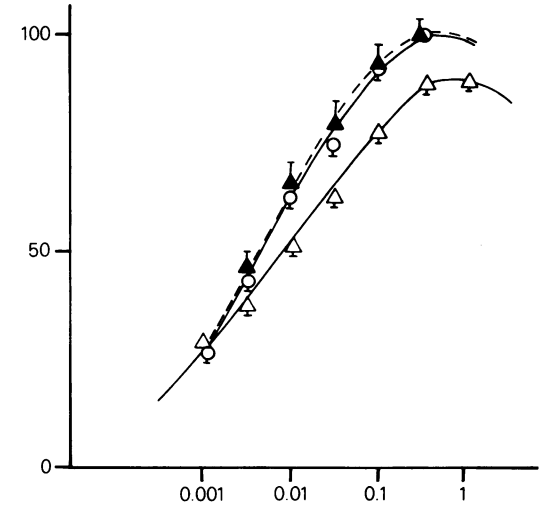

Figure 6 Guinea-pig whole ileum preparation at 12°C. Dose-response curves to histamine (○); histamine plus cimetidine, 30 μg/ml (▲) and histamine plus metiamide (△) 30 μg/ml. On the ordinate scale, percentage of the maximum response; on the abscissa scale, doses in μg/ml. Each value refers to the mean of the values obtained from 4 to 8 experiments. Vertical bars are standard errors.

histamine and this inhibitory effect which was very similar at both 37°C and 12°C (Figure 5) was not blocked by cimetidine or tiotidine (up to 30 μg/ml). Figure 6 which refers to the whole ileum preparation shows again the dose-response curve to histamine at 12°C together with the lack of any inhibitory effect of cimetidine (30 μg/ml); furthermore it also shows the antagonism exerted by high concentrations of metiamide which appeared to be of the non-competitive type as shown by the depression of the maximum response to histamine (−11.4%; $P<0.02$ by Student's t test). Tiotidine (not shown), behaved in the same way as cimetidine. In the longitudinal muscle strip the depression of the maximum response after metiamide administration was not significant ($P>0.1$ by Student's t test) (Figure 7). As shown in Figure 8 in this preparation the dose-response curve to 2-aminoethylthiazole obtained at 12°C was not modified by cimetidine (30 μg/ml) but was shifted to the right by chlorpheniramine (3 ng/ml).

Discussion

Our data do not provide any evidence for a temperature-dependent interconversion of histamine H_1- into H_2-receptors. Histamine and the relatively selective H_1-agonist, 2-aminoethythiazole were both more active at 12°C than at 37°C on the whole ileum and especially on the longitudinal muscle. Differences were statistically significant

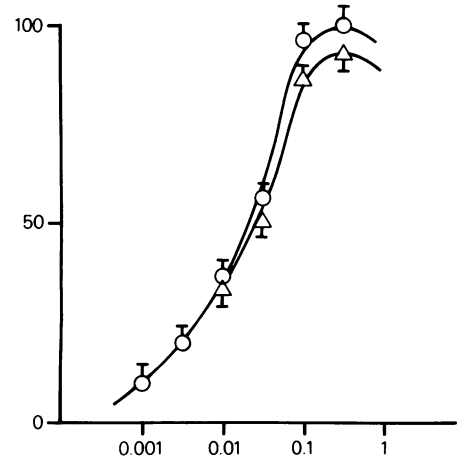

Figure 7 Longitudinal muscle strip preparation from the guinea-pig ileum at 12°C. Dose-response curve to histamine (○) and to histamine plus metiamide (30 μg/ml) (△). On the ordinate scale, percentage of the maximum response; on the abscissa scale, doses in μg/ml. Each value refers to the values obtained from 4–6 experiments. Vertical bars are standard errors.

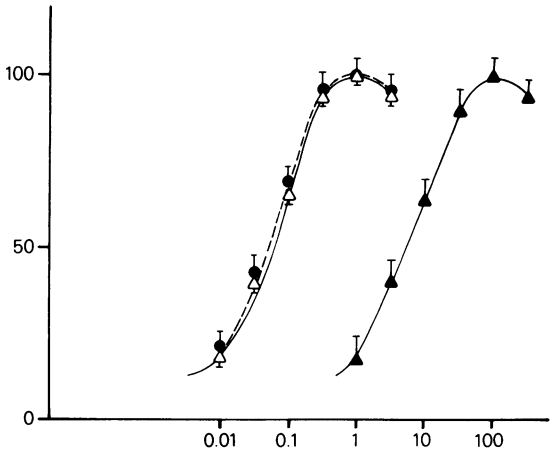

Figure 8 Guinea-pig ileum longitudinal muscle strip at 12°C. Dose-response curves to 2-aminoethylthiazole (△), to 2-aminoethylthiazole plus cimetidine, 30 μg/ml (●) and to 2-aminoethylthiazole plus chlorpheniramine, 3 ng/ml (▲). On the ordinate scale, percentage of the maximum response; on the abscissa scale, doses in μg/ml. Each value represents the mean of the values obtained from 5 experiments. Vertical bars are standard errors.

($P<0.05$) at the maximum doses. However, though several hypotheses may be suggested for this behaviour (mixed action on smooth muscle and on nervous structures with a denervation-supersensitivity, depression of enzymatic degradation etc.) it is unlikely that a transformation of H_1- into H_2-receptors may be involved in this phenomenon. Moreover histamine and 2-aminoethylthiazole were similarly antagonized at both temperatures by the H_1-receptor antagonist, chlorpheniramine. On the other hand H_2-receptor agonists, dimaprit and impromidine were always devoid of stimulatory effects up to concentrations higher than 30 μg/ml. They exerted a relaxant effect on the contraction elicited by histamine at 12°C having the same behaviour at 37°C and thus confirming our previous results obtained in the guinea-pig duodenum (Bertaccini & Zappia, 1981). Moreover, cimetidine was ineffective at 12°C exactly as it was at 37°C again confirming some previous observations (Bertaccini, Molina, Zappia & Széli, 1979). In a few preliminary experiments the new H_2-blocker, tiotidine, was similarly ineffective (up to 30 μg/ml). Conversely, metiamide (30 μg/ml) exerted a certain antagonism, apparently of the non-competitive type since it caused a slight but significant depression of the maximum response to histamine of the whole ileum. The effect of metiamide on the response to histamine was even less evident on the longitudinal muscle strip and the depression of the maximum response was not statisti-

cally significant. Metiamide was ineffective against acetylcholine-induced contractions. This could be due to the high concentrations which had to be used (lower concentrations were absolutely inactive) with the consequent possible appearance of non-specific effects of the compound. Non-specific effects of all the available H_2-receptor antagonists of the older and the newer types have already been reported (Domschke & Domschke, 1980; Bertaccini & Dobrilla, 1980; Bertaccini & Coruzzi, 1981). The fact that similar results were obtained in the whole ileum and in the longitudinal muscle strip preparation tends to minimize the possible role of the circular smooth muscle in all the observed phenomena. In conclusion, our results conflict with those of Kenakin *et al.* (1974) who claimed that at 12°C, histamine H_1-receptors of the guinea-pig ileum were transformed into H_2-receptors. However they made this assumption only on the basis of the antagonism elicited by metiamide which they found to be a competitive one. Our experiments performed by the use of different antagonists but also different agonists of the H_2-receptors seem to exclude the possibility that the low temperature converts the classical H_1-receptors of the guinea-pig ileum into a form similar to the H_2-receptors. Even though differences in Ringer solutions and method of recording responses may be partly responsible for the discrepancy between our data and those of Kenakin *et al.* (1974), we would like to emphasize, as has been previously claimed (Bertaccini, 1978), that the occurrence of the H_2-receptors may be established with certainty only: (a) if this effect of histamine is mimicked by H_2-selective agonists; (b) if this effect is competitively inhibited by H_2 selective antagonists; and (c) if the effect is not mimicked and not inhibited by the H_1-agonists and H_1-antagonists respectively. It is of interest that recent studies (Robinson & Horst, 1981) performed on the rabbit mesenteric artery strip allowed the authors to conclude that cooling-enhanced increase in histamine-induced relaxation, does not result from histamine H_2-receptor-stimulation. Similar findings were reported by Tripathi & Tayal (1980) who studied the guinea-pig atrium and observed the same degree of competitive antagonism of cimetidine on the histamine dose-response curve at both 37°C and 21°C, whereas mepyramine was ineffective at both temperatures. They concluded that no temperature-dependent interconversion of histamine H_1- and H_2-receptors occurs in the guinea-pig atrium.

This work was supported by a grant from the Smith Kline & French S.p.A., Milano, Italy.

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(Received February 26, 1982.
Revised September 14, 1982.)