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Effect of Histamine H_2-Receptor Antagonists on Acute Inflammatory of the Rat Paw Oedema

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Abstract—The effect of three histamine H_2-antagonists, cimetidine, ranitidine, and loxtidine, on acute rat paw oedema induced by histamine, carrageenan or complete Freund's adjuvant, have been examined. Administered intraperitoneally, all three antagonists inhibited histamine-induced paw oedema dose-dependently in the range 0·5–15 μmol kg^{-1}. The highest dose of cimetidine produced an inhibition of 92% as against 38% with the same dose of mepyramine. Analysis of the concentration-effect curves produced IC50 values of 1·66, 5·12 and 12·30 μmol kg^{-1} for cimetidine, loxtidine, and ranitidine, respectively, on histamine-induced oedema. In carrageenan-induced inflammation 12·3 μmol kg^{-1} of each of the three drugs produced significant inhibition, whereas in adjuvant-induced inflammation, (acute phase), cimetidine was very active, loxtidine less so and ranitidine inactive. Thus the relative effectiveness of the antagonists (cimetidine > loxtidine > ranitidine) appears to differ from their known potency relationship (loxtidine > ranitidine > cimetidine) on H_2-mediated effects. We conclude that H_2-receptors are involved in the induction of rat paw oedema, especially those induced by histamine and carrageenan, but that their relative effectiveness appears atypical.

Following the discovery of histamine H_1 and H_2-receptor subclasses and the development of specific antagonists (Black et al 1972), it has been established that in man and experimental animals, the pro-inflammatory effect of histamine may be mediated via both H_1 and H_2-receptors (Gallant et al 1973; Marks & Greaves 1977; Owen & Woodward 1980). However, species variations in the relative contribution of one receptor over the other have been reported. For example, while Owen et al (1980) reported a predominantly H_1-mediated histamine effect on cutaneous vascular permeability in guinea-pigs, Woodward & Ledgard (1986), working with hamsters, and Al-Haboubi & Zeitlin (1981), working with rats, reported a predominantly H_2-mediated effect on those species. Further studies by Al-Haboubi & Zeitlin (1982), showed in rats that cimetidine (a histamine H_2-receptor antagonist) inhibited both histamine-induced acute paw oedema and chronic adjuvant arthritis. However, in most of these studies cimetidine was used as the antagonist. This drug is known to possess many actions that are independent of H_2-receptor blockade and hence not shared by other H_2-receptor antagonists. These include binding to cytochrome P450, (Wallin et al 1979), anti-androgenic effect (Funder & Mercer 1979) and immunological activities (Avella et al 1978; Plaut & Lichtenstein 1982).

We therefore set out to determine if the anti-inflammatory action of cimetidine in rat paw oedema is shared by the other H_2-receptor antagonists—ranitidine and the new long-acting drug, loxtidine (Brittain et al 1982). The rank order of potency of the drugs was also determined and compared with their established order in H_2-mediated effects to further examine the specificity of the actions. The study was carried out using three models of acute rat paw oedema induced by histamine, carrageenan and complete Freund's adjuvant.

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Materials and Methods

Animals

Male albino rats of Wistar strain, 100–200 g, freely supplied with food and water and maintained at 26 ± 1°C, were used.

Evaluation of inflammatory responses

Acute foot paw inflammatory oedema was induced by a subplantar injection into the left hind paw of 0·1 mL of the following agents: histamine acid phosphate 2 mM, complete Freund's adjuvant or 2% w/v carrageenan in sterile 0·9% w/v NaCl (saline). The same volume of saline was injected into the right hind paw to serve as control. Paw diameter was measured 'blind', using vernier callipers accurate to 0·01 mm, always at the same point on the paw. Paw swelling in histamine-induced oedema was monitored either at 10 min intervals for 40 min, or as a single measurement after 30 min. With the carrageenan and adjuvant oedema, measurement was made at 1 h intervals for 6 h.

Pre-treatment with the histamine antagonist mepyramine, cimetidine, ranitidine or loxtidine was by injecting 0·2 mL of the appropriate dose of a drug intraperitoneally 2 h before induction of inflammation.

Drug

Histamine acid phosphate, ranitidine hydrochloride (Glaxo), loxtidine hemisuccinate (Glaxo), and carrageenan were made up in saline; complete Freund's adjuvant (Difco) was used as supplied.

Analysis of results

Except in histamine-induced oedema, results were analysed as an area under the curve of effect vs time graph. Since the responses are slow and progressive this analysis is preferred as it takes care of any skew in the peaking of the responses.

Areas under the curves were determined by calculating the number of square units enclosed by the curves and the

abscissa, the scale of the graphs being constant for any group of studies. Statistical comparison of the differences between groups receiving different treatments was with Student's *t*-test. Statistical significance was taken at $P \leq 0.05$.

Results

Effect of cimetidine, ranitidine and loxtidine on histamine-induced paw oedema

Injection of 0·1 mL of 2 mM histamine into the rat paw produced an immediate swelling which reached a maximum of about 40% increase within 30 min and declined thereafter. Saline injected into the contralateral paw produced an increase of about 5% within the same time.

Fig. 1 shows the effect of the three histamine H_2-antagonists, cimetidine, ranitidine and loxtidine as well as an H_1-receptor antagonist, mepyramine on histamine-induced paw oedema. The three H_2-antagonists inhibited paw swelling in a dose-dependent manner in the dose range 0·5–15·0 μmol kg^{-1}. The IC50 values were calculated to be 1·66, 5·12 and 12·30 μmol kg^{-1} for cimetidine, loxtidine and ranitidine, respectively, producing a potency ratio of 1:3·1:7·4. In terms of maximum effect achieved with the highest dose tested (15 μmol kg^{-1}), cimetidine produced an inhibition of 92% compared with 76% by loxtidine and 54% by ranitidine. Although at the same dose, mepyramine produced a relatively small (but statistically significant) inhibition of 38%, at lower doses its effect was comparable with those produced by same doses of loxtidine and ranitidine.

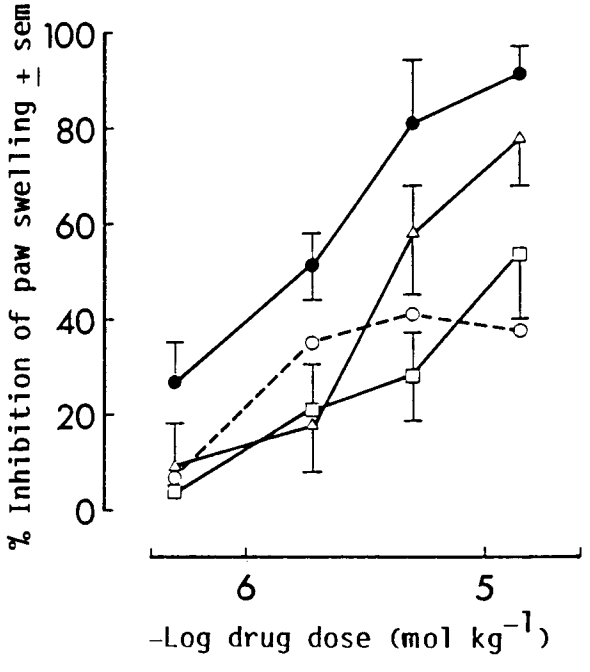

FIG. 1. Dose-response relationship of the inhibition of histamine (2 mM)-induced foot paw swelling by the three H_2-antagonists-cimetidine (●), loxtidine (△) and ranitidine (□) as well as the H_1-antagonist-mepyramine (○). Single measurements of the paw swelling made 30 min after induction were used for the analysis. Saline-induced swelling in contralateral paws were subtracted before percentage inhibition was calculated. Drugs were administered intraperitoneally 2 h before induction of inflammation. Points are means and bars are standard errors, n = 4–6.

Effect of H_2-receptor antagonists on carrageenan-induced paw swelling

Subplantar injection of 2% carrageenan resulted in an intense acute inflammatory paw swelling which progressed rapidly, reaching a peak of 160–180% increase in paw diameter in about 3 h (Fig. 2a).

To compare the effects of the three H_2-antagonists on this model a single dose of 12·3 μmol kg^{-1} was used. This dose is the IC50 of the least active of the three drugs in the histamine model. Results in Fig. 2a, b show that pre-treatment with all the three H_2-receptor antagonists, as well as mepyramine, resulted in pronounced inhibition of the paw oedema. When the results were quantitated as areas under the effect vs time curve, it was found that equimolar doses of cimetidine, ranitidine and loxtidine produced inhibitions of 70·6% (from 165·8 ± 18·3 to 48·7 ± 10·6 units^2), 50·7% (from 165·8 ± 18·3 to 81·6 ± 16·6 units^2) and 77·3% (from 165·8 ± 18·3 to 37·6 ± 8·2 units^2), respectively. Compared with the non-treated animals these reductions were statistically significant at $P < 0.001$, 0·05 and 0·001, respectively.

Mepyramine, at the same dose, produced a statistically significant inhibition of 58·3%.

Effect of H_2-receptor antagonists on adjuvant-induced paw swelling

Injection of complete Freund's adjuvant into rat foot pad produced, within the first 6 h, an inflammatory paw swelling somewhat similar to that seen with carrageenan except in the rate of increase and the magnitude. In this model, increase in swelling continued without peaking over 6 h. These measurements were made to give an increase of about 78%—half that with carrageenan (Fig. 3a). Pretreatment with equimolar doses of the antagonists showed that ranitidine and loxtidine produced only moderate inhibitions (reductions in area under the time course curve) of 27·5% (131·2 ± 11·0 to 95·1 ± 7·1 units^2) and 31·3% (from 131·2 ± 11·0 to 90·1 ± 9·4 units^2), respectively, the effect of loxtidine reaching statistical significance at $P < 0.05$. Cimetidine again produced a pronounced inhibition of 69·1% (from 131·2 + 11·0 to 40·5 ± 3·4 units^2), see Fig. 3b.

Unlike in the histamine and carrageenan models, mepyramine did not produce a significant effect in this model (21·2%). The small inhibitions produced by ranitidine, loxtidine and mepyramine occurred during the 3rd–6th h).

A summary of the relative effectiveness of the three H_2-receptor antagonists on the three types of acute paw inflammation gives the following order: Histamine: cimetidine > loxtidine > ranitidine; carrageenan: cimetidine = loxtidine ≥ ranitidine; complete Freund's adjuvant (early phase): cimetidne ≫ loxtidine > ranitidine.

Discussion

In rats, and perhaps many other species, increased cutaneous vascular permeability has been shown to be mediated predominantly by H_2-receptors (Al-Haboubi & Zeitlin 1979, 1981; Woodward & Ledgard 1986). Since cimetidine was used in most of these studies, we attempted to determine the effect of newer H_2-receptor antagonists, and whether H_2-receptors (and by implication histamine) are involved in the different forms of acute rat paw inflammation.

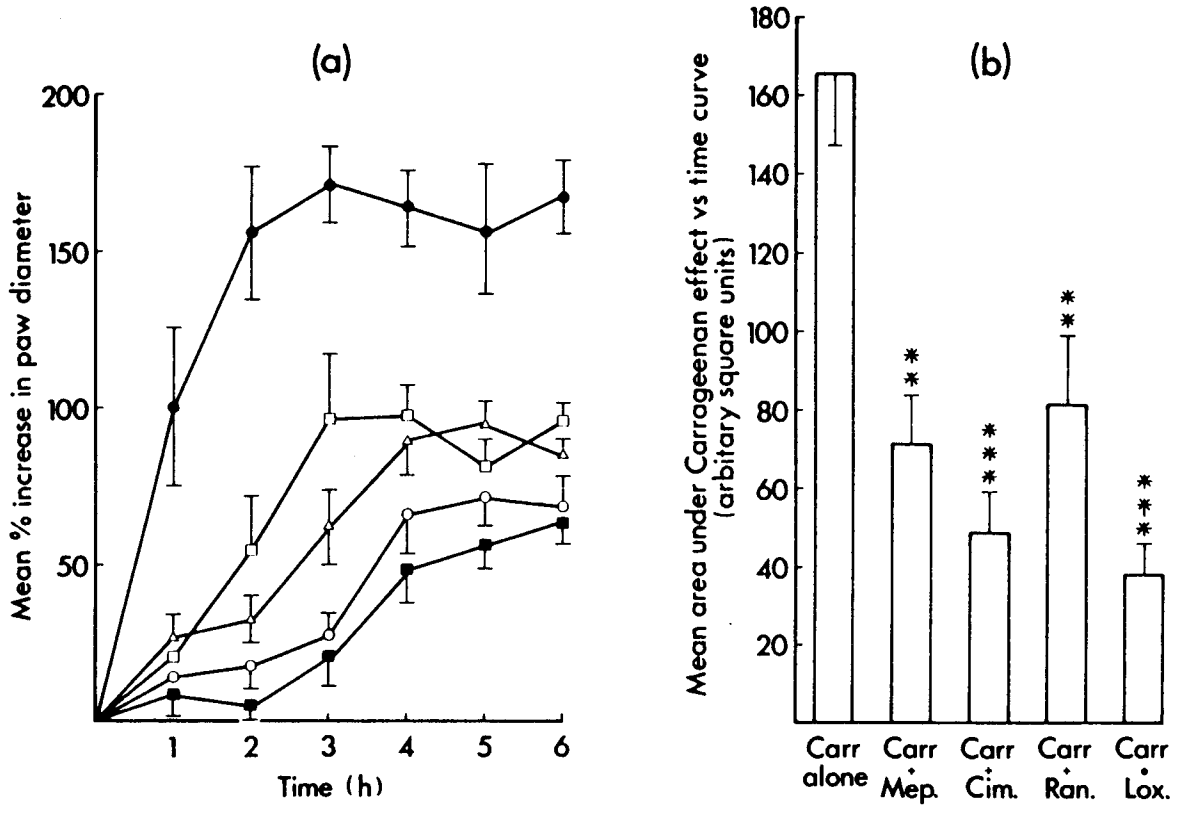

FIG. 2. Effect of H_2-antagonists and mepyramine on carrageenan-induced foot paw oedema. (a) Time-course of percentage increase in paw swelling in untreated animals (●), and in animals pre-treated with cimetidine (○), ranitidine (△), loxtidine (■) and mepyramine (□). All drugs were given at a dose of 12·3 μmol kg^{-1} intraperitoneally 2 h before induction of inflammation. (b) Areas under the curves (a) expressed as arbitrary units^2. Histograms are means for 5 or 6 curves (representing 5 or 6 animals) for each drug. ***$P < 0.001$, **$P < 0.001$.

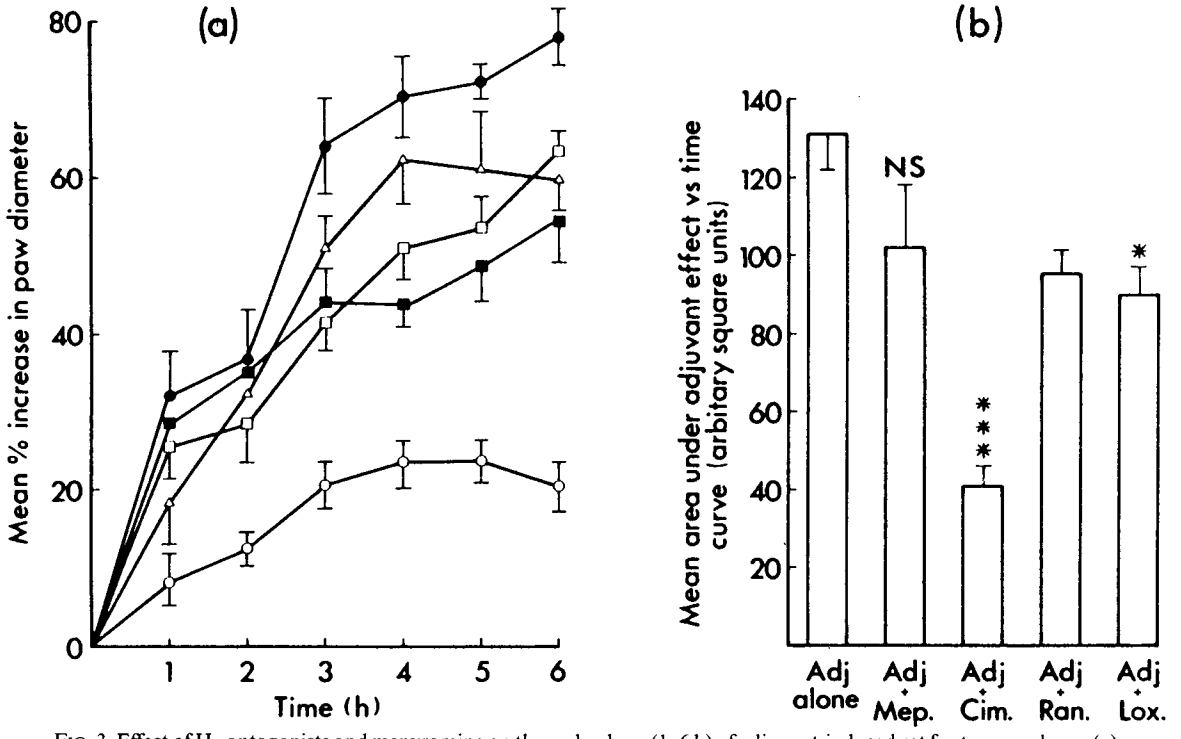

FIG. 3. Effect of H_2-antagonists and mepyramine on the early phase (1–6 h) of adjuvant-induced rat foot paw oedema. (a) Time-course of percentage increase in paw swelling in untreated animals (●), and in animals pre-treated with cimetidine (○), ranitidine (□), loxtidine (□) and mepyramine (△). All drugs were given intraperitoneally at a dose of 12·3 μmol kg^{-1} 2 h before induction of inflammation. (b) Area under the curves in (a) expressed in arbitrary units^2. Histograms are means of 5–6 different curves (5–6 animals) for each drug, and comparisons are made with controls. ***$P < 0.001$, *$P < 0.05$.

Our findings show that in rat paw oedemas, especially those induced by histamine itself, or carrageenan, H_2-receptors play a role. Also, an H_1-mediated component appears to be present as demonstrated by the significant inhibition produced by the typical H_1-receptor antagonist mepyramine.

That all the three H_2-antagonists effectively inhibited histamine-induced oedema suggests that this action may not be unique to cimetidine as is the case with androgen-receptor binding (Funder & Mercer 1979) and cytochrome P450 binding (Wallin et al 1979), but may probably be a truly H_2-mediated effect. Nevertheless, cimetidine was consistently and rather surprisingly more potent than both loxtidine and ranitidine, being 3 times more potent than loxtidine and 7·5 times more potent than ranitidine. Although mepyramine appeared weak when compared with cimetidine in histamine-induced oedema, it was of comparable potency with loxtidine and ranitidine.

For the other forms of inflammatory paw oedema, the number of animals required for the construction of full dose-response curves (which would have allowed potency ratio comparisons to be made) precluded this approach and led to one dose of 12·3 μmol kg^{-1} being used, since it was the dose at which each drug had been determined to produce at least 50% inhibition in the histamine model.

In the carrageenan model, cimetidine and loxtidine appeared equiactive, with ranitidine being much less so, while in the adjuvant model cimetidine was much more active than loxtidine and ranitidine, the latter appearing inactive in this model. Mepyramine produced statistically significant inhibition of carrageenan-induced, but not adjuvant-induced oedema. However, in neither case was its effect significantly different from those of loxtidine and ranitidine.

The relative effectiveness of the three H_2-receptor antagonists was: cimetidine > loxtidine > ranitidine. However, this is different from the documented order of potency of the three drugs acting on the H_2-receptor. In inhibiting most H_2-mediated actions such as heart rate and gastric acid secretion, loxtidine is 2–4 times more active than ranitidine which is 5–10 times more active than cimetidine, depending on the preparation (Konturek et al 1980; Brittain & Jack 1983). The reason for this reversal of potency, especially between cimetidine and ranitidine, in the carrageenan model is not known.

If it is assumed that the drugs were acting via typical H_2-receptors, then it becomes possible to see cimetidine as possessing an unusually high anti-inflammatory effect—the additional effect probably being mediated through a non-H_2 mechanism. Viewed in this context, loxtidine and ranitidine would then appear to maintain their usual order of potency. Situations in which cimetidine produces effects unrelated to H_2-receptor activation are known, (Funder & Mercer 1979; Wallin et al 1979; Plaut & Lichtenstein 1982).

Another possible implication would be that since the potency of mepyramine was not significantly different from that of loxtidine and ranitidine in all the models, both H_1 and H_2-receptors are probably involved to equal extents in the mediation of the drugs actions. Another observation is the comparative usefulness of the carrageenan model over the adjuvant model in the study of histamine-mediated inflammatory processes. The pronounced inhibition of carrageenan-induced oedema by the three antagonists as against the generally less pronounced effect in the adjuvant model, suggests that histamine release is a major component in the mechanism of the former but not the latter. This is further supported by the fact that mepyramine is an effective inhibitor of carrageenan, but not adjuvant, paw oedema. Significant histamine release by carrageenan has been reported (Capasso et al 1975). Perhaps, the only anomaly in the above analysis is that cimetidine was equally active against adjuvant-induced oedema, but again this is consistent with the drug acting through an additional non-H_2-mediated mechanism.

It is concluded that while there is good evidence that H_2-antagonists inhibit some rat paw oedemas through H_2-receptor blockade, it is not certain whether the H_2-receptors involved are atypical or whether cimetidine possesses an unusual additional anti-inflammatory effect unrelated to H_2-blockade.

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