

# Anti-IgE-Induced Histamine Release from Human Basophilic Leukocytes: Inhibition Depends on Nature and Concentration of Inhibitor and Secretagogue

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Histamine release from human basophilic leukocytes was induced by increasing concentrations of anti-IgE in the presence of various concentrations of 2-deoxyglucose (2-DOG), the histamine H<sub>2</sub>-receptor agonist dimaprit, theophylline, or enprofylline (a new antiasthmatic xanthine derivative). The results show that the degree of inhibition produced by each agent differed with the concentration of anti-IgE used and with the nature and concentration of the inhibitor. These data indicate that great care should be used when characterizing an inhibitor of mediator release by simply giving a figure for the per cent inhibition of release observed in its presence.

*Key words:* basophilic leukocytes; 2-deoxyglucose; dimaprit; enprofylline; histamine release; theophylline.

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## CLINICAL ASPECTS

Studies with 2-deoxyglucose, the histamine H<sub>2</sub>-receptor agonist dimaprit, theophylline, and enprofylline indicate that the degree of inhibition exerted by a specific inhibitor at release of histamine from human basophils induced by anti-IgE depends on i) the degree of stimuli used ii) the nature of the inhibitor and iii) its concentration. These findings might have implications for studies of the effect of antiallergic agents at the clinical level.

Studies of agents with potential antiallergic capacity usually include the examination of their inhibitory effects in a number of mediator release systems *in vitro*. It is then customary to express an inhibitor's effect by giving the concentration that produces 50% inhibition of the degree of release, as seen in its absence. Several literature reports make it clear that this way of expressing inhibitory capacity of an agent is not fully adequate. Thus, e.g., at antigen-induced histamine release from chopped guinea pig lung,  $\beta$ -stimulating agents seem capable of shifting antigen dose-response curves to the

right (i.e. reducing cell "sensitivity" in the terms of Zeiss et al. (11)) and of reducing maximum degree of histamine release attainable (i.e. reducing cell "reactivity" (11)) by independent processes (12). Furthermore, the capacity of the antiallergic xanthine derivative D 4026 to inhibit histamine release from human basophilic leukocytes apparently depends on the degree of basal release induced (7). Recently, Tung & Lichtenstein (10) showed that cyclic AMP agonist inhibition increases at low levels of histamine release from human basophils. The present report examines the inhibitory effect at anti-IgE-induced histamine release from human basophils of four different agents;

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2-deoxyglucose (2-DOG), dimaprit (a histamine  $H_2$ -agonist), theophylline, and enprofylline (a principally new antiasthmatic xanthine (6)). Concentration-response curves were constructed for anti-IgE-induced histamine release at various concentrations of each agent. The results indicate that the observed degree of inhibition depends on both the nature and the concentration of the inhibitor examined and also on the concentration of the secretagogue used to induce release.

## EXPERIMENTAL PROCEDURES

Anti-IgE-induced release of histamine from basophilic leukocytes was performed and quantified as previously described in detail (7) but at slightly modified buffer conditions. In brief, blood from healthy donors was allowed to sediment in a mixture of dextran-EDTA and glucose. After 45–60 min, the leukocyte-rich supernatant plasma was withdrawn and the cells were washed once with HEPES-A buffer containing 5.96 g HEPES, 7.01 g NaCl, 0.37 g KCl, and 0.3 g human serum albumin per liter, pH 7.4 (25°C). The washed cells were finally resuspended in HEPES-ACM buffer containing the above-mentioned constituents together with 88.2 mg  $CaCl_2 \times 2 H_2O$  and 210 mg  $MgCl_2 \times 6 H_2O$  per liter buffer solution. Histamine release experiments were performed in a 1.2 ml sample. The number of leukocytes per sample varied with experimental conditions. The cells were preincubated at 37°C for 10 min together with inhibitor at the specified concentration or buffer before challenge with anti-IgE. The reaction was allowed to proceed for 40 min. All samples were run in duplicate. Histamine secreted into the supernatant was assayed by spectrophotofluorimetry (7). The amount of histamine released into the supernatant was expressed as per cent of total cellular histamine determined after lysis of cells with perchloric acid.

The anti-IgE used was prepared and purified as described (7). This preparation was diluted in HEPES-ACM buffer to the final dilutions indicated in the text. Sources of chemicals used: 2-deoxyglucose (grade III), HEPES (N-2-

hydroxyethylpiperazine- $N'$ -2-ethane sulfonic acid), human serum albumin, and theophylline were obtained from Sigma; dimaprit was kindly provided by Dr. G. J. Durant, Smith, Kline & French Lab. Ltd., Welwyn Garden City, UK. Enprofylline was kindly synthesized by Dr. G. Kjellin, AB Draco. Inhibitors were solubilized in HEPES-ACM buffer just before use. There was no indication that any of the agents used should interfere with the assay of histamine.

## RESULTS

The present work examines anti-IgE-induced histamine release from human basophilic leukocytes obtained from a number of different donors. It is well known that cells from different individuals vary with respect to release capacity at a given concentration of anti-IgE. This is also our experience. Fig. 1 illustrates dose-response curves for three different individuals. Each of these individuals was examined on three or more occasions. Obviously, some individuals respond well to anti-IgE (as individual No. 1), some are less good responders (as individual No. 3), and some individuals respond badly to low anti-IgE concentrations, but reach better responsiveness at high anti-IgE concentrations (as individual No. 2). Most of our donors belong to category No. 1.

### *Effect of dimaprit on anti-IgE-induced release*

Parallel dose-response curves for anti-IgE-induced release of histamine were performed in both the presence and the absence of dimaprit. Table 1 shows the results recorded in five experiments at  $10^{-5}$  M dimaprit. A plot of degree of release in the presence of inhibitor versus the degree of release in its absence is shown in Fig. 2A. A slightly curvilinear relation between the two parameters is indicated. A straight line was fitted to the data pairs plotted in Fig. 2A by the method of least squares and to similar data pairs recorded at  $10^{-6}$  M,  $10^{-4}$  M and  $10^{-3}$  M dimaprit (see Fig. 2B). Clearly, a biphasic pattern of inhibition for dimaprit is suggested by these regression lines. In a first phase, at very low degrees of control release, a high degree of

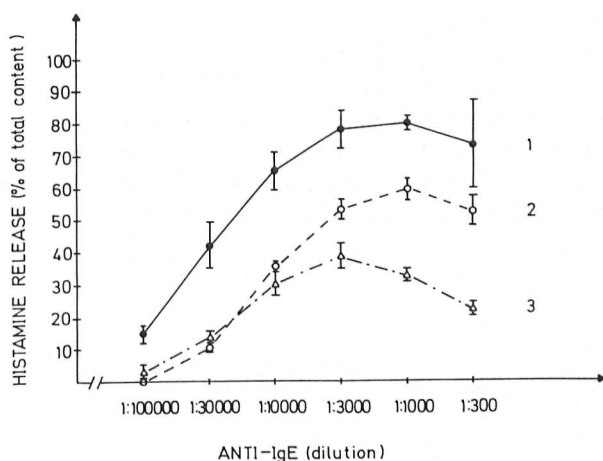


Fig. 1. Mean degree of histamine release (expressed in per cent of total cellular histamine content) at the indicated dilutions of anti-IgE using cells from three different individuals (No. 1, 2, and 3). The individuals were examined at three (No. 1), three (No. 2), or four (No. 3) different occasions. Bars indicate SEM.

Table 1

Histamine release (expressed in per cent of total cellular histamine content) at the indicated final concentrations of anti-IgE with cells from four different individuals (one individual was examined twice) in the presence of vehicle (v) or  $10^{-5}$  M dimaprit (DIM). Figures in brackets give degree of inhibition of release

| Indiv. No. |     | Anti-IgE dilution |         |         |         |         |         |
|------------|-----|-------------------|---------|---------|---------|---------|---------|
|            |     | 1:100000          | 1:30000 | 1:10000 | 1:3000  | 1:1000  | 1:300   |
| 3          | v   | 1                 | 10      | 32      | 42      | 32      | 20      |
|            | DIM | 0                 | 9 (10)  | 19 (41) | 20 (52) | 17 (47) | 9 (55)  |
| 10         | v   | 18                | 40      | 54      | 62      | 64      | 67      |
|            | DIM | 3 (83)            | 17 (57) | 32 (41) | 42 (32) | 45 (30) | 46 (31) |
| 2          | v   | 0                 | 11      | 39      | 54      | 63      | 54      |
|            | DIM | 0                 | 2 (82)  | 7 (82)  | 30 (44) | 36 (43) | 36 (33) |
| 6          | v   | 6                 | 27      | 47      | 55      | 51      | 34      |
|            | DIM | 1 (83)            | 7 (74)  | 17 (64) | 28 (49) | 19 (63) | 11 (68) |
| 3          | v   | 9                 | 20      | 39      | 49      | 38      | 28      |
|            | DIM | 2 (78)            | 10 (50) | 20 (49) | 24 (51) | 18 (47) | 12 (57) |

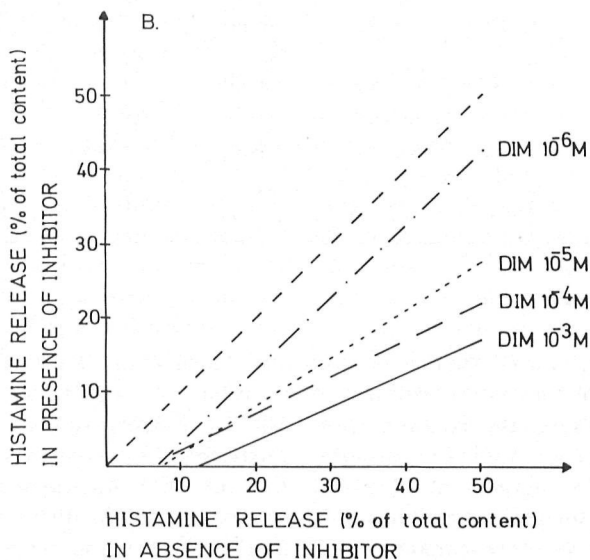
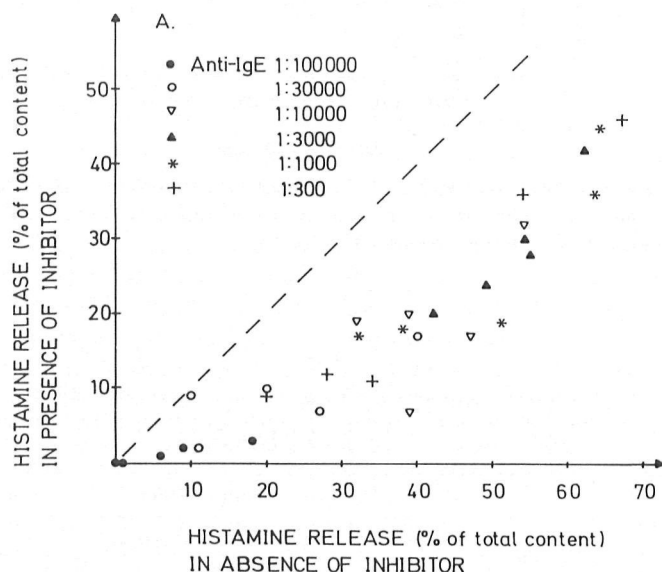
inhibition is seen (see Fig. 2A). Then, as control release increases, inhibition is proportional to degree of control release, the factor of proportionality being dependent on the concentration of dimaprit. This agrees with Fig. 2C where median values for per cent inhibition of release are plotted versus concentration of anti-

IgE. Clearly, the degree of inhibition depends on inhibitor concentration, but  $10^{-5}$ ,  $10^{-4}$ , and  $10^{-3}$  M dimaprit do not vary markedly in effectiveness. The data recorded also indicated that the inhibitory capacity of a specified concentration of dimaprit varies with individuals independent of the degree of release obtained

(Fig. 3). Thus, with cells from individual No. 3 (a "low" responder to anti-IgE), dimaprit at  $10^{-6}$  M or at  $10^{-5}$  M is not more efficient as an inhibitor than it is with cells from individual No. 10 (a "high" responder to anti-IgE). On the other hand, dimaprit at  $10^{-6}$  M barely affects release of histamine from cells of individual No. 11.

### *Effect of 2-DOG on anti-IgE-induced histamine release*

We examined, for six individuals, histamine release at different anti-IgE concentrations in the presence of  $3 \times 10^{-4}$  M 2-DOG or vehicle. Included in this experiment were individuals No. 1 and 2 of Fig. 1, i.e., individuals of dif-



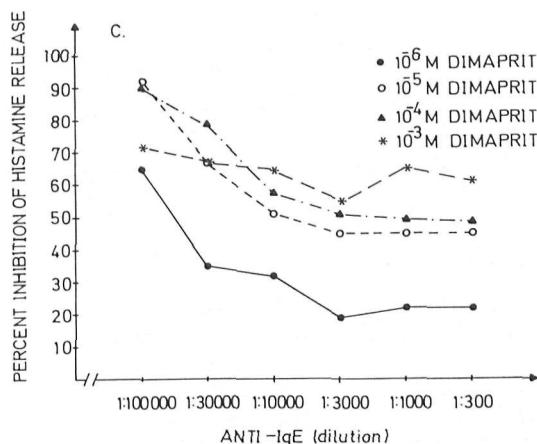


Fig. 2A. A plot of degree of histamine release (expressed in per cent of total cellular histamine content) in the presence of dimaprit at  $10^{-5}$  M versus that observed in its absence at challenge with anti-IgE of varying degrees of dilution. Data from Table 1.

B. Regression lines fitted to data pairs recorded for degree of release in the presence and in the absence of dimaprit at various concentrations of anti-IgE. The equations for the regression lines were the following: At dimaprit  $10^{-6}$  M  $y = -6.8 + 0.97x$  ( $n = 30$ ), at dimaprit  $10^{-5}$  M  $y = -4.6 + 0.64x$  ( $n = 30$ ), at dimaprit  $10^{-4}$  M  $y = -2.8 + 0.50x$  ( $n = 30$ ), and at dimaprit  $10^{-3}$  M  $y = -5.8 + 0.46x$  ( $n = 24$ ).

C. Degree of inhibition of histamine release by different concentrations of dimaprit at the indicated concentrations of secretagogue. Each point represents median values of per cent inhibition registered for each of the examined individuals. Per cent inhibition for each defined concentration of secretagogue and inhibitor was calculated for each examined individual by dividing degree of release in the presence of inhibitor with degree of release in its absence, subtracting the resulting figure from 1, and multiplying with 100.

Test according to Kruskal-Wallis showed that significant differences existed between groups examined at different inhibitor concentration at anti-IgE 1:30000, 1:10000, 1:3000, and 1:1000 ( $P < 0.05$ ), but not at anti-IgE 1:100000 or 1:300.

ferent response categories were represented. Table 2 gives the results. These clearly indicate that, irrespective of the individual examined, the degree of inhibition observed at a given concentration of 2-DOG depends much more on the concentration of secretagogue used than on the degree of release induced. This is evident when degree of release in the presence of inhibitor is plotted versus degree of release in its absence (Fig. 4). Therefore dose-response curves were recorded for anti-IgE-induced release of histamine at the chosen concentrations of secretagogue in the presence of different concentrations of 2-DOG. For each concentration of 2-DOG, at least five experiments were performed, but no effort was made to choose individuals of a specified category according to Fig. 1. With no 2-DOG, 29 experiments with cells from 11 individuals were performed. For each concentration of 2-DOG and anti-IgE,

median values of inhibition of release were determined. Fig. 5 shows results expressed as per cent inhibition. These results show that 2-DOG at  $10^{-6}$  M,  $10^{-5}$  M and  $3 \times 10^{-5}$  M partly inhibit histamine release induced by low anti-IgE concentrations, but do not affect release at high anti-IgE concentrations, that 2-DOG at  $10^{-4}$  and  $3 \times 10^{-4}$  M completely inhibit release at low anti-IgE concentration, but only slightly affect release at high anti-IgE concentration, and that 2-DOG at  $10^{-3}$  M and  $3 \times 10^{-3}$  M completely inhibit release at low anti-IgE concentrations and partly at high.

#### *Effects of theophylline and enprofylline on anti-IgE-induced release*

Effects of theophylline and enprofylline were examined at a more restricted panel of anti-IgE concentrations than that used with the above-

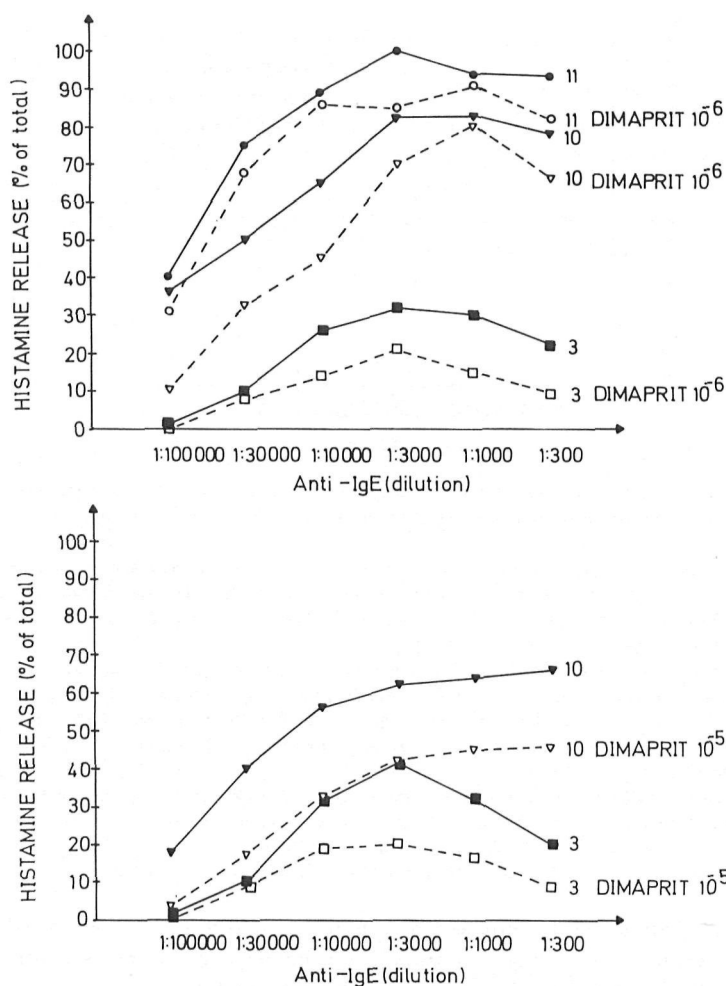


Fig. 3. Dose response curves for inhibition of anti-IgE-induced release of histamine from human leukocytes by dimaprit ( $10^{-6}$  M, frame A, and  $10^{-5}$  M, frame B) with cells from individuals 3, 10, and 11.

mentioned inhibitors. Parallel dose-response curves for anti-IgE-induced release were constructed in the presence and in the absence of drug. Fig. 6 shows the results; a few negative values (i.e., indicating slight stimulation of release by the xanthine) are put at zero. At effective concentrations, both theophylline and enprofylline equally inhibit release at each of the examined concentrations of the secretagogue; possibly both agents stimulate release at low anti-IgE concentrations rather than inhibit it. However, this finding is not significant.

## DISCUSSION

The present report provides data indicating that the degree of inhibition exerted by a specified inhibitor at release of histamine from basophils depends on the degree of stimuli used to induce release, the nature of the inhibitor, and its concentration. Furthermore, the characteristics of different inhibitors, i.e., dimaprit, 2-DOG, and methylxanthines, differed in this respect.

Table 2

Histamine release (expressed in per cent of total cellular histamine content) at the indicated final concentrations of anti-IgE with cells from six different individuals in the presence of vehicle (v) or  $3 \times 10^{-4}$  M 2-DOG. Figures in brackets give degree of inhibition of release

| Patient No. |       | Anti-IgE dilution |         |         |         |         |         |
|-------------|-------|-------------------|---------|---------|---------|---------|---------|
|             |       | 1:100000          | 1:30000 | 1:10000 | 1:3000  | 1:1000  | 1:300   |
| 2           | v     | 0                 | 12      | 34      | 47      | 53      | 44      |
|             | 2-DOG | 0                 | 0 (100) | 2 (92)  | 10 (79) | 28 (47) | 40 (9)  |
| 4           | v     | 24                | 48      | 62      | 69      | 54      | 60      |
|             | 2-DOG | 1 (96)            | 5 (90)  | 18 (71) | 40 (42) | 45 (17) | 42 (30) |
| 1           | v     | 12                | 52      | 73      | 90      | 84      | 97      |
|             | 2-DOG | 0 (100)           | 4 (92)  | 22 (70) | 60 (33) | 92 (-8) | 96 (1)  |
| 5           | v     | 26                | 50      | 60      | 57      | 57      | 45      |
|             | 2-DOG | 1 (96)            | 6 (88)  | 23 (62) | 41 (28) | 40 (30) | 31 (31) |
| 6           | v     | 41                | 74      | 93      | 93      | 83      | 65      |
|             | 2-DOG | 2 (95)            | 6 (92)  | 30 (68) | 64 (31) | 72 (13) | 51 (22) |
| 7           | v     | 56                | 98      | 128     | 115     | 113     | 95      |
|             | 2-DOG | 5 (91)            | 11 (89) | 53 (59) | 73 (37) | 96 (15) | 69 (27) |

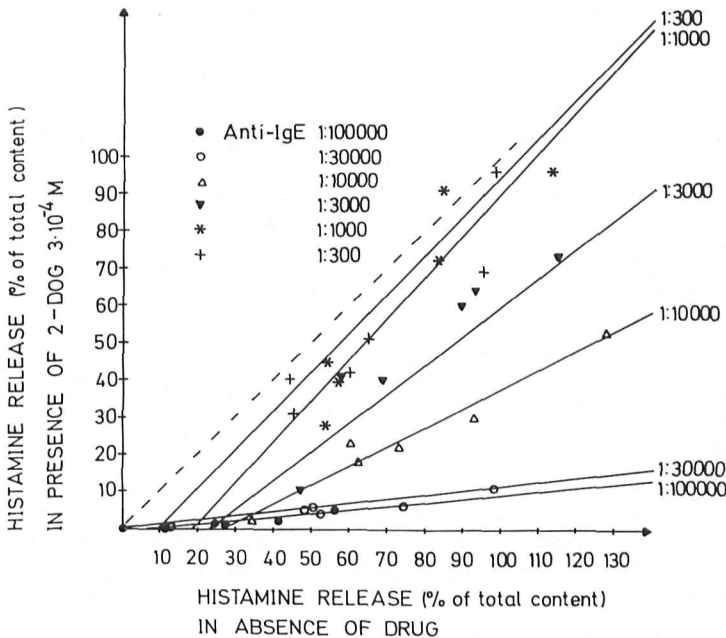


Fig. 4. A plot of degree of histamine release (expressed in per cent of total cellular histamine content) in the presence of 2-DOG at  $3 \times 10^{-4}$  M versus that observed in its absence at challenge with anti-IgE of varying degrees of dilution. Data with cells from six different individuals. Regression lines have been fitted to the data pairs of each anti-IgE dilution.

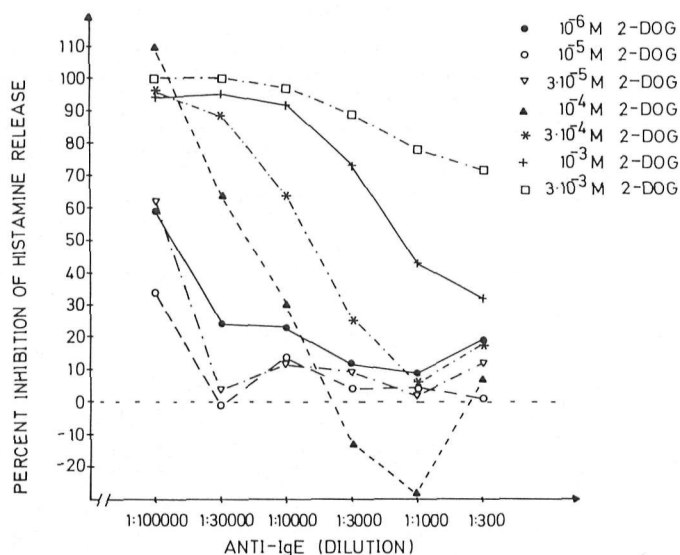


Fig. 5. Inhibition of histamine release, induced by anti-IgE at various concentrations, by increasing concentrations of 2-DOG. Each point represents median values of per cent inhibition registered for each of the examined individuals. Per cent inhibition for each defined concentration of secretagogue and inhibitor was calculated for each examined individual as described in legend to Fig. 1.

Test according to Kruskal-Wallis showed that significant differences existed between inhibitor concentrations at all examined concentrations of secretagogue ( $P < 0.05$ ).

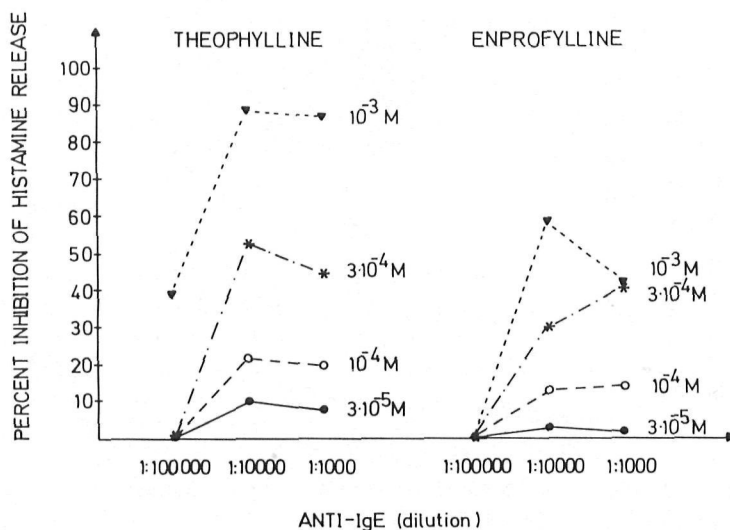


Fig. 6. Inhibition of histamine release, induced by anti-IgE at various concentrations, by increasing concentrations of theophylline and enprofylline. Inhibition of release was calculated as described in legend to Fig. 2. Each point is based on data from four to six experiments except for those obtained at 1:100000 anti-IgE which are based on three experiments.

Concerning dimaprit, our data corroborate those recently published by Tung & Lichtenstein (10). However, there is no clear indication in the data of Tung & Lichtenstein (10) that inhibition exerted by dimaprit should follow a biphasic pattern, as suggested by the Fig. 2 data. The biologic reason for this finding is clearly intriguing but cannot yet be defined. It could be argued that the use of dimaprit is not ideal in studies such as the present one, as cells from some individuals seem to respond to this agent by increased histamine release rather than by its inhibition (9). Furthermore, as shown in Fig. 3, the inhibitory capacity of a specified concentration of dimaprit might vary with individuals, independent of the induced degree of histamine release. These facts might explain why our data with dimaprit less clearly show decreased inhibitory effect of the compound with increased degree of stimulation than do those of Tung & Lichtenstein (10). A further possible reason for the slight discrepancies observed is the difference in secretagogue used; dimaprit is more effective as an inhibitor of antigen-induced than anti-IgE-induced histamine release (cf. 4). Furthermore, Tung & Lichtenstein used cells from allergic individuals, cells which might be affected by endogenous levels of plasma histamine (cf. 8) whereas we used cells from apparently normal individuals.

Concerning 2-DOG, the following findings seem worth pointing out: The inhibitory capacity of 2-DOG clearly depends on a combination of dose of inhibitor and concentration of secretagogue used to induce release; in fact, even more than we find that of dimaprit to be. Thus; such an effect is not restricted to cyclic AMP active agents as was implied by the data of Tung & Lichtenstein (10). With 2-DOG, release induced by low concentrations of anti-IgE is more easily affected by the inhibitor, and then apparently by unknown reasons irrespective of the degree of release induced, than is release induced by high anti-IgE concentration (Figs. 4, 5). Again, this is an intriguing finding. At low concentrations of anti-IgE, cells from most individuals release only small amounts of histamine, but cells from some individuals – due to a high

degree of releasability? – respond well. The biological mechanism determining the degree of release at this condition is apparently more sensitive to inhibition by 2-DOG than is the mechanism determining cell reactivity. The reason for this is not clear.

We (7) and Tung & Lichtenstein (10) also found a negative correlation between the degree of inhibition recorded for the xanthines D 4026 and 3-isobutyl-1-methylxanthine (IBMX), respectively, and the degree of release. However, the negative correlation noted by Tung and Lichtenstein with 3-isobutyl-1-methylxanthine was of less magnitude than those seen with dimaprit and PGE<sub>1</sub>. Ennis et al. (2) reported a similar situation for theophylline-induced inhibition of histamine release from rat mast cells stimulated by compound 48/80 or peptide 401. In previous (unpublished) experiments performed with the Tris-buffer system previously used (7), we also noted such an effect with theophylline, but in the present experiments, we see no such correlation between degree of basal release and effect of theophylline or enprofylline with the human basophil system. The reason for this is not clear; our change of buffer system could contribute, but other reasons should also be considered. Thus, the present findings cannot be taken as an indication that theophylline or enprofylline exert inhibition of histamine release from basophils without influencing levels of cyclic AMP.

Finally, the important pharmacological implication of the present type of findings for tests of inhibitory capacity of antiallergic agents have already been pointed out (7, 10). Inhibition of antigen- or compound 48/80-induced release of histamine from rat peritoneal mast cells by DSCG, inhibition of antigen-induced histamine release from actively sensitized chopped guinea pig lung by  $\beta$ -stimulating agents and methyl xanthines, and inhibition of 7S<sub>2</sub>-mediated passive cutaneous anaphylactic reactions by DSCG, apparently follow the same general rule, i.e. with increased stimuli, inhibition is less expressed (see refs. 1, 2, 5, and our own unpublished observations). This pattern of decreased relative effect of an inhibitor with increased degree of stimuli is not apparently

limited to the mediator release process; inhibition of antigen-induced contractions of guinea pig tracheal strips by isoproterenol is also characterized by this kind of relation (1). A similar effect pattern has been described for PGE<sub>2</sub>-induced inhibition of PHA-stimulated (<sup>3</sup>H) thymidine incorporation of lymphocytes (3). Therefore, the existence of this kind of relation between the degree of stimulus and the degree of inhibition exerted by a given drug at a given concentration should be considered also in clinical situations, e.g. at the test of anti-allergic drugs.

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