

Desferal (desferrioxamine)—A novel activator of connective tissue-type mast cells

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The effect of Desferal (desferrioxamine), an iron-chelating agent with allergic side effects, was examined on human basophils and rodent mast cells (MCs) in vitro and in the human skin. Even at a high concentration (100 mg/ml), the drug neither induced histamine release (HR) from human basophils nor primed these cells to release higher amounts of histamine when they were activated with f-met-peptide or anti-IgE antibodies. In contrast, in all seven subjects studied, intradermal injection of Desferal (0.1 mg/ml to 100 mg/ml) elicited classic wheal-and-flare responses. Ingestion of 10 mg of cetirizine, an H₁ antagonist, 3 hours before the intracutaneous administration of Desferal, significantly reduced the diameters of both wheal-and-flare reactions, indicating that the drug caused local HR. Desferal also induced HR from rat peritoneal MCs in vitro but had no effect on mouse bone marrow-derived MCs. These results suggest that Desferal has a direct, IgE-independent, stimulatory effect on connective tissue-type MCs. Thus, it may be used as a positive control in skin testing. (J ALLERGY CLIN IMMUNOL 1991;88:854-60.)

Key words: Desferal, skin, mast cell, histamine release

Desferal (Ciba-Geigy, Basel, Switzerland) (desferrioxamine) is an iron-chelating agent widely used since the early 1960s for the treatment of iron overload in thalassemic patients and in iron intoxication. Although it is administered to many patients as continuous infusion, either intravenously or subcutaneously, only a few side effects have been reported. These include mild local erythema at the injection site, cataract and ocular toxicity.^{1,2} Systemic immediate-type allergic reactions to Desferal are relatively rare. Few cases have been reported in which patients developed anaphylactoid symptoms, such as hypotension, generalized itching, wheezing, and tachycardia, within minutes after administration of the drug.³⁻⁵ Specific IgE or IgG Abs against Desferal

Abbreviations used

Ab:	Antibody
MC:	Mast cell
TG ⁺⁺ :	Tyrodé's buffer containing 0.1% gelatin 1.8 mmol/L of CaCl ₂ , and 0.9 mmol/L of MgCl ₂
HR:	Histamine release
BMMC:	Bone marrow-derived mast cell
LPR:	Late-phase reaction

were not found, and the mechanism of action by which it causes allergic symptoms has not been investigated.

In the present study, we examined the effect of Desferal in vitro on rat peritoneal MCs, mouse BMMCs, and human basophils, and in vivo on human skin. Our results indicate a direct releasing effect of this drug on connective tissue-type MCs.

MATERIAL AND METHODS

Subjects

Seven healthy volunteers (five male and two female subjects), aged 24 to 40 years, were included in the study group after their informed consent was obtained. Two of the subjects had a history of mild allergic rhinitis. None of the

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Received for publication April 5, 1991.

Revised July 2, 1991.

Accepted for publication July 5, 1991.

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subjects had taken medication for at least 3 weeks before the study.

Skin testing

Intradermal skin tests were performed on the volar aspect of the arm with a 0.5 cc syringe with a 28-gauge needle. Twenty microliters of the following test solutions were injected: phosphate-buffered saline (negative control), histamine phosphate, 1:1000 (Eli Lilly & Co., Indianapolis, Ind.), and tenfold serial dilutions (0.01 to 100 mg/ml) of Desferal, diluted in phosphate-buffered saline (desferrioxamine methansulfonate, Ciba-Geigy). The wheal-and-flare sizes were measured after 15 minutes and recorded as the mean of two orthogonal diameter measurements in absolute values. We did not subtract the control site responses. In five subjects, an additional set of skin tests was performed 3 hours after the ingestion of a single dose (10 mg) of cetirizine (Zirtec; UCB Laboratory, Torino, Italy).

Preparation of leukocyte suspensions and basophil activation

Forty milliliters of venous blood was collected and anticoagulated with 0.01 mol/L of ethylenediaminetetraacetic acid. The blood sample was mixed with 6% dextran in saline and 30 mmol/L of dextrose and allowed to sediment for 60 to 90 minutes at room temperature, according to the method of Lichtenstein and Osler.⁶ The leukocyte-rich plasma was centrifuged, and the cell pellet was washed twice in Tyrode's buffer, pH 7.4. The cells were then suspended in Tyrode's buffer containing TG^{++} . Basophils were counted in duplicate in a Fuchs-Rosenthal (Firma Walter Schrenck GmbH, Hoffheim am Taunus, Germany) chamber with alcian blue stain; a final concentration of 4×10^4 /ml of basophils was obtained, adjusting the volume of TG^{++} buffer. Leukocyte suspensions were stimulated in 12 by 75 mm polyethylene tubes. Briefly, 2×10^4 basophils suspended in 0.5 ml of TG^{++} were added to two tubes containing 0.5 ml of Desferal, diluted in TG^{++} (final concentration, 1 μ g/ml to 200 mg/ml) for 15 minutes at 37° C. To determine possible priming effect of Desferal on immunologic or nonimmunologic HR, partially purified basophils were incubated with different drug concentrations (1 μ g/ml to 100 mg/ml) for 5 minutes at 37° C. At the end of this preincubation period, optimal doses of human anti-IgE Abs (1:5000, Sigma Chemical Co., St. Louis, Mo.) or f-met-peptide (10 mmol/L, Sigma Chemical Co.) were added for 30 additional minutes. At the end of the incubation periods, supernatants and cells were separated by centrifugation and kept at -20° C for histamine assay.

Rat peritoneal MC activation

MCs were obtained from Sabra male rats (an outbred strain of the Hebrew University), weighing 250 to 300 gm, by peritoneal lavage and purified as previously described.⁷ The freshly isolated and purified (>95% MCs) MCs were resuspended at a density of 0.4 to 1.2×10^5 /ml in TG^{++} .

One hundred microliters of TG^{++} buffer containing different concentrations of Desferal (100 to 0.001 mg/ml) was added to the MCs for 15 minutes at 37° C. After this incubation period, supernatants were collected by sedimentation of the cell suspensions at 120 g for 5 minutes at room temperature. The cell pellets were resuspended in 200 μ l of TG^{++} buffer and were disrupted by continuous sonication for 15 seconds (output 5, 50% duty cycle, Heat Systems Ultrasonics, Inc., W380, Farmingdale, N.Y.).

Mouse BMMC activation

Mouse BMMCs were obtained by culturing bone marrow cells from BALB/c mice for 3 weeks in plastic culture flasks (Falcon Plastics, Becton Dickinson Labware, Lincoln Park, N.J.) with 50% enriched medium (RPMI 1640) containing 10% heat-inactivated fetal calf serum (vol/vol), 100 U/ml of penicillin, 100 μ g/ml of streptomycin, 2 mmol/L of L-glutamine, 0.1 mmol/L of nonessential amino acids, pH 7.2 (Grand Island Biological Co., Grand Island, N.Y.), and 50% WEHI-3B cell-conditioned medium, as previously described.⁸ The culture medium was changed every 7 days. After 3 weeks, >95% of the cells in the suspension cultures were MCs, as assessed by metachromatic staining with toluidine blue. BMMCs were washed twice by sedimentation with TG^{++} and resuspended at a concentration of 1×10^6 /100 μ l of TG^{++} buffer; 100 μ l of TG^{++} buffer containing various concentrations of Desferal (100 to 0.001 mg/ml) was added, and the cells were incubated for 20 minutes at 37° C. After this incubation time, cell suspensions were processed as described for rat peritoneal MCs.

Cell viability assay

To assess MC viability after activation, cultures were incubated for 5 minutes at room temperature with a solution of 0.4% trypan blue (Sigma Chemical Co.) in saline. The percentage of stained cells was determined by counting 100 consecutive cells.

Mediator assays

As a marker of cell activation, histamine was determined in the supernatants and in the cell sonicates of rat peritoneal MCs and human basophils, whereas β -hexosaminidase was determined in the supernatants and in the cell sonicates of mouse BMMCs.

Histamine was assessed by a radioenzymatic assay with ¹⁴C-labeled methyl-S-adenosyl-L-methionine (41 mCi/mmol/New England Nuclear, Boston, Mass.) and a crude preparation of rat kidney methyl transferase.⁹ The assay was calibrated with solutions containing 0 to 10 ng of histamine (histamine dihydrochloride, Sigma Chemical Co.). β -Hexosaminidase activity was quantified by the release of p-nitrophenyl- β -2-acetamido-2-deoxyglucopyranoside in a reaction mixture containing 4 mmol/L of substrate in 0.5 mmol/L of citrate buffer.¹⁰ The percentages of histamine and β -hexosaminidase released by the MCs and ba-

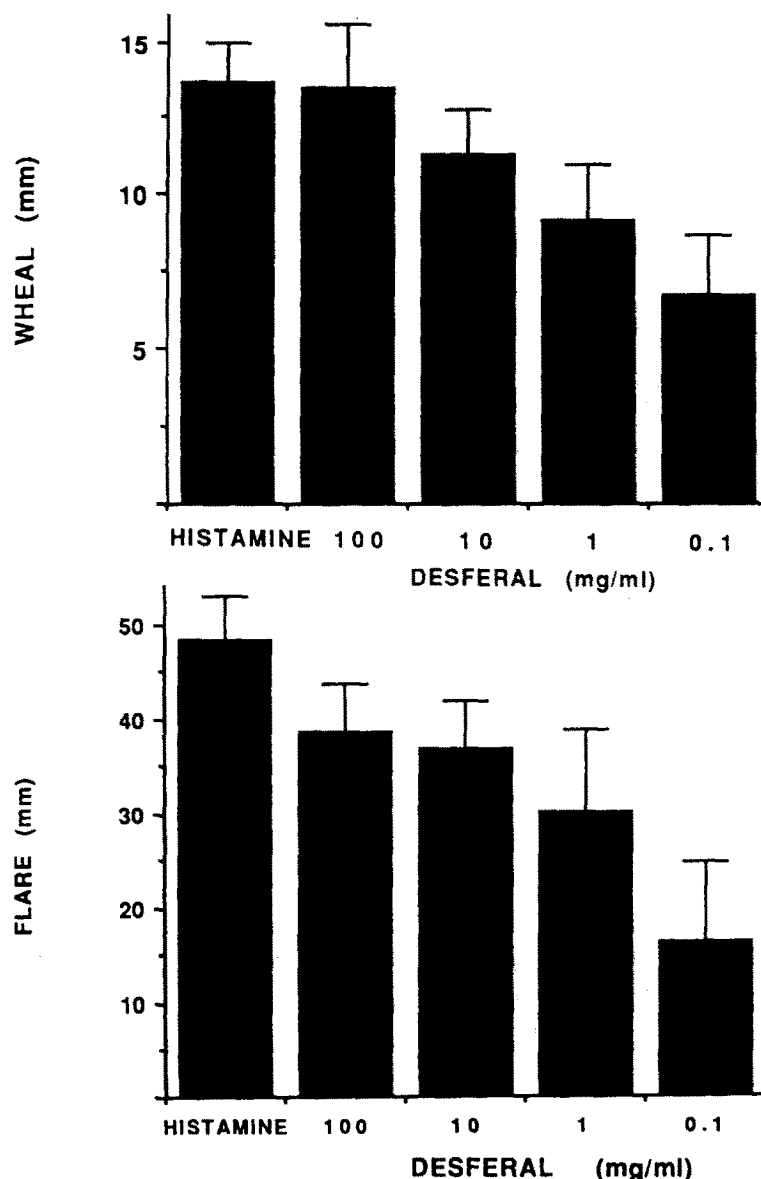


FIG. 1. Desferal-induced wheal-and-flare responses. Intradermal skin test responses to histamine (1:1000) and Desferal (0.1 to 100 mg/ml) in seven subjects. Results are expressed as mean $\pm$ SD of wheal-and-flare sizes.

sophils incubated with buffer and with the different concentrations of Desferal were calculated by dividing the amount of the mediator in the supernatants by the sum of that in the supernatants and the respective sonicated cells.

Statistical analysis

Data are expressed as mean $\pm$ SD. All the in vitro experiments were performed at least in duplicate. Results were analyzed by the Student's *t* test.

RESULTS

HR from human basophils

In vitro incubation of human basophils with increasing concentrations of Desferal (1 μ g/ml to 10 mg/ml) failed to induce HR from these cells. During the incubation period, >98% of basophils remained

viable, as determined by trypan blue exclusion. At a concentration of 100 mg/ml, Desferal caused an HR of $15.1\% \pm 7.2\%$, but this result was most likely due to cytotoxic effect of the drug, since cell viability decreased to $89.3\% \pm 3.2\%$ ($n = 7$). At higher concentrations (>150 mg/ml) of Desferal, >40% of the basophils were not viable. Preincubation with Desferal (1 μ g/ml to 10 mg/ml) had no effect on HR from basophils stimulated with optimal concentrations of antihuman IgE Abs or f-met peptide ($n = 5$).

Cutaneous wheal-and-flare reactions

In all seven subjects studied, Desferal induced classic immediate-type cutaneous hypersensitivity responses manifested as typical wheal-and-flare reac-

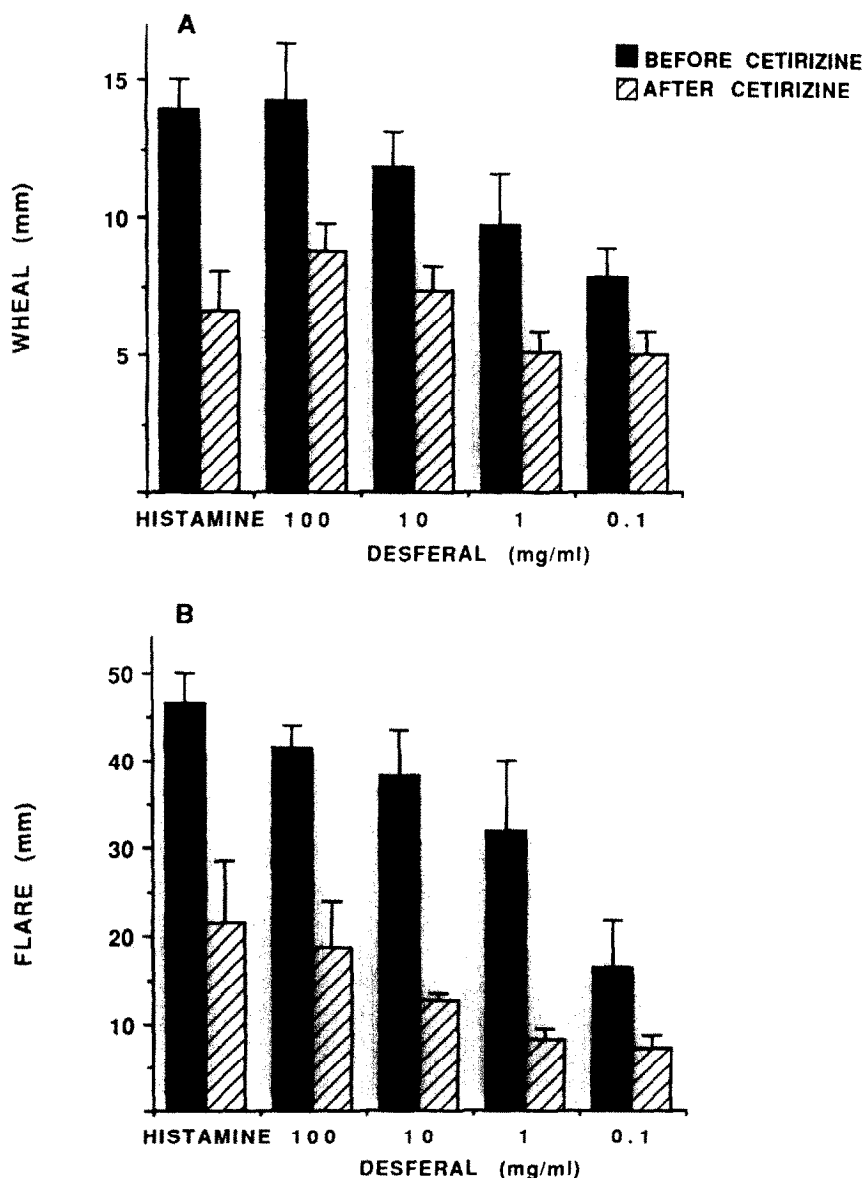


FIG. 2. Wheal-and-flare size before and after cetirizine administration. **A**, Wheal and **B**, flare responses (mean $\pm$ SD) to histamine and Desferal in five subjects before and 3 hours after ingestion of 10 mg of cetirizine.

tion, reaching maximal size 15 minutes after skin testing. The mean wheal-and-flare diameters at the highest Desferal concentration (100 mg/ml) skin test sites were 13.5 ± 2.1 mm and 39.7 ± 5.1 mm, respectively (Fig. 1). These diameters were comparable to the mean wheal size (13.6 ± 1.4 mm) and the mean flare size (48.4 ± 4.6 mm) induced by histamine. When lower concentrations of Desferal were injected at different skin sites, the diameters of both wheals and flares diminished progressively in a dose-response dependent fashion (Fig. 1). At sites of Desferal concentrations of 0.01 mg and lower, the mean dimension of wheals did not differ from dimensions of wheals of buffered-saline diluent (negative control) test sites, which were between 2 and 4 mm. Careful

inspection of Desferal skin test sites for 48 hours in all subjects did not reveal any signs of cutaneous LPR.

Effect of cetirizine on skin tests

To evaluate whether the cutaneous wheal and flare observed at skin sites 15 minutes after intradermal injection of Desferal was due to mediator release from local MCs, five subjects were studied before and 3 hours after ingestion of cetirizine, an H_1 -blocker drug.

As illustrated in Fig. 2, after cetirizine administration, the mean diameters of both wheal and flare at the histamine sites (positive controls) were significantly reduced ($p < 0.001$ and $p < 0.005$ for wheal-and-flare sizes, respectively), indicating that the drug

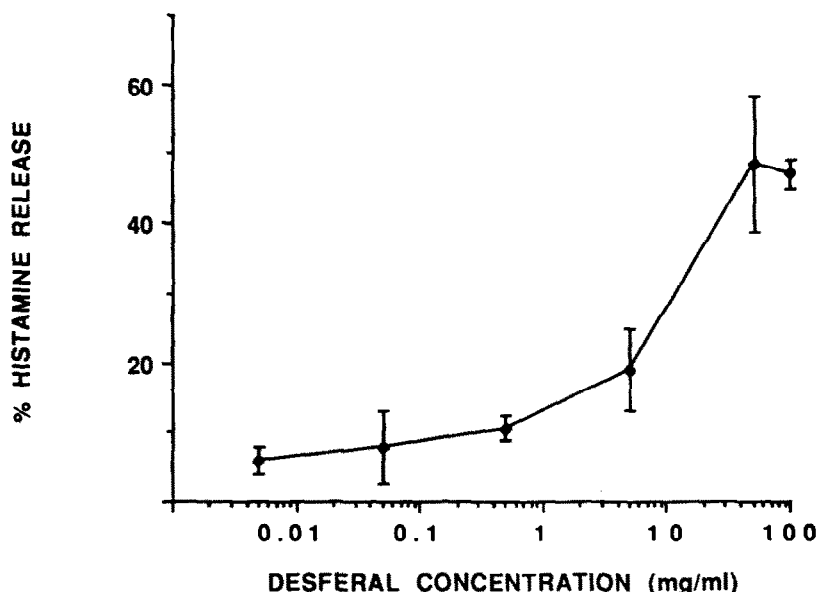


FIG. 3. Percentage of Desferal-induced HR from rat peritoneal MCs. MCs incubated with various concentrations of Desferal. These data represent the mean $\pm$ SD of experiments performed in duplicate.

inhibited local effects of histamine. Ingestion of cetirizine reduced the sizes of wheals and flares induced by Desferal in each of the five individuals studied. This inhibitory effect of the antihistaminic drug was significant at all concentrations of Desferal tested, that is, at 100 to 0.1 mg/ml (Fig. 2). Highest reduction of mean wheal diameter (48%) and of mean flare diameter (74%) was observed at the 1 mg/ml skin site.

HR from rat peritoneal MCs

To determine possible direct stimulatory effect of Desferal on rodent connective tissue-type MCs, we incubated freshly isolated rat peritoneal MCs with various concentrations of the drug. At a concentration of 100 mg/ml and 50 mg/ml, Desferal induced a substantial HR of $47.1\% \pm 1.2\%$ and $48.2\% \pm 7.8\%$, respectively ($p < 0.005$ compared to buffer-incubated cells). At 5 mg/ml, there was still a significant HR ($18.9\% \pm 5.9\%$; $p < 0.05$) that gradually decreased (Fig. 3). Compound 48/80 (3 μ g/ml), used as a positive control, induced release of $32.1\% \pm 5.2\%$ histamine. In all Desferal concentrations tested, the MCs remained viable ($>96\%$), as determined by trypan blue exclusion and cell counts.

Stimulation of mouse BMMCs with Desferal

Incubation of mouse BMMCs with increasing concentrations of Desferal (5 μ g/ml to 100 mg/ml) for 15 minutes did not induce activation of this type of MC ($n = 4$). In fact, no measurable amounts of

β -hexosaminidase (a marker of mouse BMMC degranulation) could be detected in the supernatants. Desferal concentrations were not cytotoxic to the mouse BMMCs, as determined by trypan blue exclusion ($>95\%$ viable cells). In contrast, incubation of mouse BMMCs with dinitrophenol-human serum albumin after presensitization of cells with IgE anti-dinitrophenol Abs (positive control) elicited $33.5\% \pm 4.9\%$ HR.

DISCUSSION

MC activation and subsequent mediator release can be induced by IgE-dependent or IgE-independent mechanisms. Immunologic, IgE-dependent stimulation is caused by specific antigens, anti-IgE Abs, lectins, and other agents that cross-link two or more adjacent IgE molecules.¹¹ Nonimmunologic activation is carried out by a variety of substances like neuropeptides, compound 48/80, and various basic compounds.¹² These agents stimulate the MCs by binding to putative receptors (other than IgE) or through non-specific direct activation.^{12, 13}

Drugs can induce immediate-type allergic reactions in humans by several mechanisms. Penicillins and cephalosporins, acting as haptens, and insulin and chymopapaine, which are complete antigens, stimulate the mast cells by IgE-dependent mechanism.¹⁴ In contrast, opiates induce direct, nonimmunologic MC activation.¹⁵ In addition, allergic responses can be induced by activation of basophils by drugs, such as dextran and contrast media.¹⁴

In the present study we intended to investigate whether the allergic reactions caused by Desferal can be attributed to IgE-dependent or to IgE-independent mechanisms and whether the mediators released derive from activated MCs or basophils.

Human basophils incubated with increasing concentrations of Desferal were not activated to release histamine. In addition, preincubation with the drug had no priming effect on the cells to release higher amounts of histamine on challenge with immunologic or nonimmunologic stimulation. It therefore appears that basophils are not playing a role in Desferal-induced allergic reactions. Thus, prophylactic treatment with corticosteroids before the administration of Desferal, as used in the preparation of patients sensitive to radiocontrast media, will not help in reducing the allergic symptomatology, since this kind of pretreatment is effective only for drugs causing basophil degranulation.

In contrast, intradermal injection of Desferal caused a dose-dependent wheal-and-flare reaction in all the volunteers studied. Pretreatment with the antihistaminic drug cetirizine resulted in marked attenuation of the wheal-and-flare responses. This finding would indicate that histamine is released locally by cutaneous MCs and that the dermal responses observed were not merely the result of nonspecific irritation of the skin. Since the subjects in our study were exposed to Desferal for the first time in their lifetime and since the drug induced the classic wheal-and-flare response in all of the subjects, this finding would suggest a direct, nonimmunologic stimulatory effect on cutaneous MCs. Nevertheless, it is conceivable that Desferal stimulates some other cell populations to release histamine releasing factors that activate MCs.

Penicillins and cephalosporins are the major drugs inducing immunologic IgE-dependent reactions. Specific serum IgE Abs are found in many penicillin-sensitive patients, and skin tests are positive in all of these patients because of the presence of MC membrane-bound specific IgE.¹⁶ In fact, the failure to detect specific IgE or IgG Abs against Desferal in sera of patients who developed allergic symptoms to the drug⁵ would also support a nonimmunologic mechanism.

Administration of penicillin to penicillin-sensitive individuals can be done after routine rush desensitization procedures in which multiple incremental doses of the drug are administered. This mode of treatment would probably be ineffective in cases of Desferal allergy, since the mechanism of Desferal-induced activation is nonimmunologic.

These data presented herein demonstrate that Desferal does not stimulate isolated human basophils to

release histamine but induces direct, nonimmunologic activation of dermal MCs. These features are similar to features displayed by opiates. Intradermal injections of codeine or morphine cause wheal-and-flare reactions in nonpreviously exposed individuals because of direct activation of MCs.¹⁷ This effect is probably mediated by a naloxone-sensitive receptor.¹⁸ Similarly, also the widely used MC secretagogue, compound 48/80, or the neuropeptide substance P have a specific activating property toward human skin MCs, whereas they have no effect on human basophils.¹⁹

Another feature of Desferal observed in this study was the failure of this drug to induce cutaneous LPR after intradermal administration in all the subjects studied. Dolovich et al.¹⁷ reported similar findings with codeine, which elicited prominent wheal-and-flare reactions that did not proceed to LPRs. In contrast, immunologic activation of skin MCs by intradermal injection of allergen or anti-IgE Abs leads to the development of LPRs in most immediate-type responses.²⁰ The difference between the capacity of various stimulants to induce LPR may be attributed to differential release of mediators from local MCs. Although immunologic activation causes release of both histamine and arachidonic acid metabolites, opiates, neuropeptides and other nonimmunologic secretagogues induce HR alone.^{21, 22}

As noted above, opiates, compound 48/80, as well as some neuropeptides, are known to specifically activate human cutaneous MCs and rodent peritoneal MCs. These two MC types belong to the connective-tissue phenotype. To examine whether Desferal is also a specific activator of rodent connective tissue-type MCs, rat peritoneal MCs and mouse BMMCs (which are immature cells that share some histochemical and biochemical properties with the mucosal-type MCs) were incubated with Desferal. We found that only connective-tissue MCs were activated by the drug in a dose-response fashion. In contrast, BMMCs were unresponsive, even at extremely high concentrations of Desferal. This lack of effect on BMMCs is not surprising, since rodent, as well as human, mucosal MCs are nonresponsive to a number of non-IgE-dependent stimuli.^{23, 24} It is notable that Desferal and some other nonimmunologic secretagogues of rat peritoneal and human MCs are all basic compounds. Thus, these agents may stimulate MCs through a similar mechanism.

Skin tests are the most widely used technique in the diagnosis of allergy. Intradermal injection of morphine or codeine, as MC activators, are usually used as positive controls in these tests. As we have demonstrated, Desferal also induces a prominent wheal-and-flare reaction in all the subjects by a nonimmu-

nologic mechanism. This drug is safe and nontoxic, and it is conceivable to use it as a positive control in the allergy clinic.

In conclusion, we have demonstrated that Desferal is a novel activator of both human and rat connective tissue-type MCs. We also suggest its use for a positive control in intradermal skin tests.

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