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# *Comparison of Histamine Release in Human Skin Mast Cells Induced by Morphine, Fentanyl, and Oxymorphone*

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**Human leukocyte and skin mast cell preparations were incubated with morphine sulfate in concentrations ranging from $1.5 \times 10^{-5}$ M to $4.5 \times 10^{-3}$ M. Skin mast cells also were incubated with oxymorphone and fentanyl in the same concentrations. Human leukocytes did not release histamine in response to any concentration of morphine. In skin mast cells, histamine release by morphine first was detected at $1.5 \times 10^{-4}$ M. Histamine release further increased at $5.0 \times 10^{-4}$ M with no incremental increase at higher concentrations. Oxymorphone and fentanyl failed to release histamine at any concentration. Histamine release by morphine required calcium but was not influenced by changes in the 1–4 mM range. Skin mast cell preparations were pretreated for 30 min in naloxone $5 \times 10^{-4}$ M and then morphine $5 \times 10^{-4}$ M was added for 30 min without removing naloxone. Naloxone neither released histamine nor inhibited morphine-induced histamine release. The release of histamine by morphine but not equimolar concentrations of fentanyl and oxymorphone indicates that histamine release by narcotics is not a nonspecific effect of high drug concentration. The failure of naloxone to inhibit morphine-induced histamine release suggests that histamine release by morphine is not dependent on opiate receptor binding or activation. These results indicate that this human mast cell preparation will be useful in further understanding the mechanism of histamine release induced by morphine and other agents. (Key words: Analgesics: fentanyl; morphine; oxymorphone. Antagonists, narcotic: naloxone. Histamine: mast cells; release.)**

IN 1939, Alam *et al.* first showed that curare released histamine in a dog skeletal muscle preparation.[1] Subsequently, many other drugs used in anesthesia have been reported to induce histamine release.[2] Morphine and related alkaloids have attracted much clinical interest because several adverse effects of these drugs, in particular, hypotension and cutaneous urtication, have been attributed to release of histamine and other mast cell mediators.

Human basophil preparations,[3,4] lung mast cells,[5] and skin mast cells[6] are stimulated *in vitro* to release histamine by immunoglobulin E (IgE). The factors controlling this release have been studied extensively, yet little is known about factors controlling histamine release induced by nonimmunologic stimuli. The few studies available have used cells or tissues from animals,[1,7–9] but recent studies show marked functional differences between mast cells of different species and tissues.[10] Thus, the relevance of these animal studies to human mast cell histamine release is questionable.

Our studies were stimulated by the need to understand mechanisms underlying drug-induced histamine release in humans. Using human skin mast cell preparations, we determined whether histamine release by narcotics is related to drug concentration, cytotoxicity, or activation of narcotic receptors on target cells.

## Methods

### Human Leukocyte Preparation

This study was approved by our Institutional Human Research Committee. Leukocyte suspensions were prepared by a method modified from Thueson *et al.*[11] In brief, 35 ml venous blood from human volunteers was drawn into a plastic syringe containing 10 ml hydroxyethyl starch (Volex®, McGaw Laboratories) and 5 ml of 0.1 M EDTA *p*H 7.20. The syringe stood upright at room temperature for 45 min, until a clear interface was evident between erythrocytes and supernatant. The leukocyte-rich supernatant was transferred to a sterile polypropylene tube (Corning 25330), washed in buffer (10 mM HEPES, 137 mM NaCl, 5 mM KCl, and human serum albumin 0.3 mg/ml), and then centrifuged at $300 \times g$ for 8 min at 4° C. The supernatant was discarded, and the leukocyte pellet was resuspended in buffer. After two additional washings in buffer, the leukocyte pellet was resuspended in the same buffer to which was added 2 mM calcium and 1 mM magnesium ($Ca^{++}Mg^{++}$ buffer) to a concentration of $10^7$ cells/ml. Basophils in a sample of the leukocyte suspension were stained with Alcian blue and counted. Basophils constituted 1–2% of the total leukocytes.

### Drug-Induced Histamine Release in Leukocytes

Morphine sulfate powder (Merck Sharp Dohme, West Point, Pennsylvania) was diluted in $Ca^{++}Mg^{++}$ buffer. Fifty microliters of each test concentration of morphine were added to duplicate tubes in an ice water bath.

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Four-hundred fifty microliters of leukocyte suspension then were added to each tube, giving a total volume of 500 μl. Final concentrations of morphine ranged from $1.5 \times 10^{-5}$ M to $10^{-2}$ M. Cells were incubated for 45 min in a 37° C water bath with resuspension of cells by tapping and shaking the tubes every 15 min. Parallel incubation of cells in buffer alone was used to determine spontaneous histamine release. Concanavalin A (Con A) (Pharmacia, Uppsala, Sweden), a known stimulus for histamine release,[12] was used as a positive control (final concentration 1.25 μg/ml). Total histamine content was determined by boiling 450 μl cell suspension plus 50 μl buffer for 10 min. All reactions were terminated by centrifuging at 800 *g* for 8 min at 4° C. The supernatants were placed in polypropylene tubes and stored at −70° C until histamine determination.

### Human Foreskin Preparation

Freshly excised infant foreskins were placed in individual tubes (Corning 25330) that contained Dulbecco's Modified Eagle Medium® (Gibco Labs) with 1% Antimicrobic Solution® (Gibco Labs). All samples were processed within 4 hs of excision. Each foreskin was spread and tacked on a Styrofoam® cutting board and divided into eight to 12 similarly sized rectangular portions with a blade. To increase the surface area for drug interaction, each piece was sectioned further into standardized 200-μm-width strands with a Sorvall® TC-2 tissue sectioner as described by Tharp *et al.*[6] Each sectioned piece was placed in a polypropylene tube and washed three times in 2 ml buffer containing 135 mM NaCl, 3.7 mM KCl, 5 mM $Na_3PO_4$, 5 mM dextrose, and 2 mM HEPES, *p*H 7.2.

### Drug-induced Histamine Release in Skin

After washing, the sectioned pieces were suspended in 1.0 ml buffer with 0.1 mg/ml human serum albumin and 2.5 mM calcium added (Ca++ HSA buffer) and incubated for 30 min in a 37° C water bath. Tissue strands were resuspended by tapping the tubes every 10 min. The supernatant was aspirated from each tube, placed in a plastic sample cup, and frozen at −70° C until assayed for histamine concentration. This initial supernatant was used to determine spontaneous pre-drug-exposure histamine release.

Morphine sulfate powder, fentanyl citrate powder (Janssen, New Brunswick, New Jersey), and oxymorphone hydrochloride powder (DuPont, Wilmington, Delaware) were diluted to final concentrations, ranging from $1.5 \times 10^{-5}$ M to $4.5 \times 10^{-3}$ M in $Ca^{++}$ HSA buffer. Naloxone hydrochloride (Dupont, Wilmington, Delaware) was diluted to $5 \times 10^{-4}$ M in the same buffer.

Each sectioned piece then was resuspended in 1.0 ml test drug or buffer (control) and incubated for 30 min at 37° C with resuspension by tube agitation every 10 min. Each drug concentration was tested in duplicate. The supernatant was aspirated and frozen until assayed. This supernatant was used to determine the drug-induced histamine release.

In four foreskins, the effect of extracellular calcium concentration on morphine-induced histamine release was studied. After obtaining spontaneous histamine release, skin pieces were incubated for 30 min with $1.5 \times 10^{-3}$ M morphine sulfate in buffer containing 1 mM to 4 mM $CaCl_2$.

The influence of naloxone pretreatment was studied in four foreskins. For these studies, after obtaining supernatant for spontaneous histamine release, duplicates were incubated in $Ca^{++}$ HSA buffer for 60 min (control), in naloxone for 60 min, in buffer for 30 min, followed by morphine for 30 min, or in naloxone for 30 min, followed by morphine for 30 min without removing the naloxone.

To determine the residual histamine content of each sectioned piece, the tissue from each tube was placed in 3 ml $Ca^{++}$ HSA buffer, boiled for 10 min, and then homogenized with a Polytron® homogenizer. After centrifugation at 800 *g* × 5 min, the supernatant was aspirated and frozen until assayed.

### Histamine Determinations

Concentrations of histamine in supernatants were determined by the double isotope radioenzymatic method of Shaff and Beaven[13] or by the automated flurometric method of Siraganian[14] (Alpkem Histamine Autoanalyzer®, Clackamas, Oregon). Preliminary studies showed identical measurable histamine concentrations with the use of either the fluorometric or radioenzymatic assay. The sensitivity of each method was 1–2 ng of histamine per milliter. Assay specificity of both methods was confirmed by incubation with diamine oxidase (Sigma, St. Louis, Missouri), which eliminated all detectable histamine. For each assay, a standard curve was generated with histamine solutions of known concentration.

In the enzymatic assay, the reaction mixture consisted of 92 μl crude rat kidney histamine methyl transferase enzyme, 5 μl S-adenosyl-L-(methyl-$^{14}$C) methionine with a specific activity of 61 mCi/mmol (Amersham, Arlington Heights, Illinois), and 3 μl of a 1:1,200 dilution of $^{3}$H-histamine that had a specific activity of 8.8 Ci/mmol (New England Nuclear, Boston, Massachusetts). The labeled histamine was used as an internal standard. Fifty-microliter samples supernatants were added to the reaction mixture, and the remainder of the assay was conducted as described by Shaff and Beaven.[13]

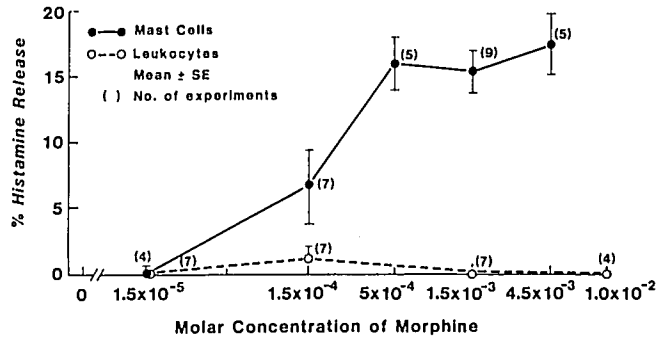


FIG. 1. Per cent histamine release from plasma leukocytes and human skin in increasing concentrations of morphine sulfate. Morphine sulfate induced dose-related histamine release from skin mast cell preparations (● —— ●) but not from leukocyte preparations (O - - - O).

Spontaneous and drug-induced histamine release were calculated as a per cent of the total histamine present. The total histamine concentration in the leukocyte preparation was determined by the histamine concentration of the supernatant of boiled cells. The total histamine in each sectioned piece of foreskin was calculated by adding the histamine concentrations in the supernatants for spontaneous histamine release, drug-induced histamine release, and residual histamine. All results are expressed as per cent of net histamine release, which was calculated by subtracting per cent of spontaneous histamine release from per cent of drug-induced histamine release. Results were discarded if the spontaneous histamine release was greater than 10% in leukocytes or 17% in skin mast cells.

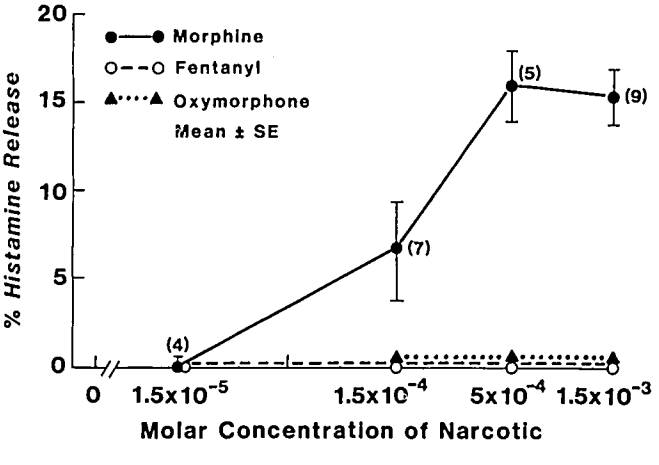


FIG. 2. Comparison of per cent histamine release from human skin *in vitro* in the presence of increasing concentrations of morphine, fentanyl, and oxymorphone. In fentanyl and oxymorphone studies, n = 3. In morphine studies n is indicated in parentheses. Morphine induced dose-related histamine release (● —— ●), whereas fentanyl (O - - - O) and oxymorphone (▲ - - - ▲) failed to release histamine at any concentration used.

### CYTOTOXICITY STUDIES IN SKIN

To determine if histamine release by morphine was the result of nonspecific cell membrane damage, lactic dehydrogenase (LDH) was measured with the use of a Gilford® spectrophotometer (Oberlin, Ohio) in six separate foreskin experiments. Foreskins each were divided into three pieces, sectioned as previously described, and incubated in buffer for 30 min at 37° C. LDH was measured and assigned a relatively activity of 100%. One of the three pieces was incubated in buffer (negative control), one in water (positive control), and one in morphine $1.5 \times 10^{-3}$ M for an additional 30 min, and LDH and histamine were measured. LDH was expressed as per cent change from the LDH measured after the first incubation.

### STATISTICAL ANALYSIS

Data were compared by a one-way analysis of variance and a Student Newman-Keuls test for multiple comparison. The level of statistical significance used was $P < 0.05$.

## Results

In leukocyte preparations, spontaneous histamine concentrations were usually 5–15 ng/ml and total histamine concentrations 100–200 ng/ml. Mean spontaneous histamine release was 3.0 ± 0.4% (mean ± SEM). In skin mast cell preparations, spontaneous histamine concentrations ranged from 5 to 35 ng/ml and total histamine concentrations ranged from 100 to 300 ng/ml. Mean spontaneous histamine release was 7.9 ± 0.5% (mean ± SEM).

Morphine sulfate induced dose-related histamine release from skin but not leukocyte preparations (fig. 1). In leukocyte preparation, drug incubation caused no histamine release, regardless of concentration. In contrast, histamine release by Con A (positive control) was 22.1 ± 8.5% (mean ± SEM).

In skin preparations, morphine $1.5 \times 10^{-5}$ M failed to induce histamine release above spontaneous levels. Morphine $1.5 \times 10^{-4}$ M induced histamine release of 4.9 ± 3.1%, which was significantly greater than control preparation, as well as release measured at $1.5 \times 10^{-5}$ M morphine ($P < 0.05$). Histamine release further increased to 15.8 ± 2.0% at $5 \times 10^{-4}$ M morphine. This per cent release was significantly greater than histamine release with $1.5 \times 10^{-4}$ M morphine ($P < 0.05$). Higher concentrations of morphine did not result in further histamine release.

Incubation of skin mast cells with the same concentrations of fentanyl and oxymorphone failed to induce release of histamine above spontaneous levels at any concentration (fig. 2).

Morphine-induced histamine release required calcium. In the absence of calcium in the incubating buffer, histamine release induced by $1.5 \times 10^{-3}$ M morphine was reduced to 5% above spontaneous. However, calcium concentrations from 1–4 mM produce similar histamine release by morphine. In the presence of 1, 2, 3 and 4 mM $Ca^{++}$ $1.5 \times 10^{-3}$ M morphine produced 14.9 ± 3.3% (mean ± SEM), 18.1 ± 3.4%, 13.0 ± 2.7%, and 18.2 ± 2.2% histamine release, respectively ($P$ NS).

Morphine $5 \times 10^{-4}$ M produced similar histamine release in the presence and absence of $5 \times 10^{-4}$ M naloxone (16.9 ± 4.0% and 17.1 ± 2.8%, respectively). Naloxone alone produced no histamine release (fig. 3).

Morphine sulfate $1.5 \times 10^{-3}$ M did not increase LDH activity indicating that morphine induced histamine release was not due to a cytotoxic mechanism. LDH decreased to 55 ± 10% (mean ± SE) after 30 min incubation with morphine, which was not significantly different from LDH measured after incubation with buffer alone (75 ± 12% decrease). In contrast, incubation with water (positive control) increased LDH 203 ± 32% ($P < 0.05$).

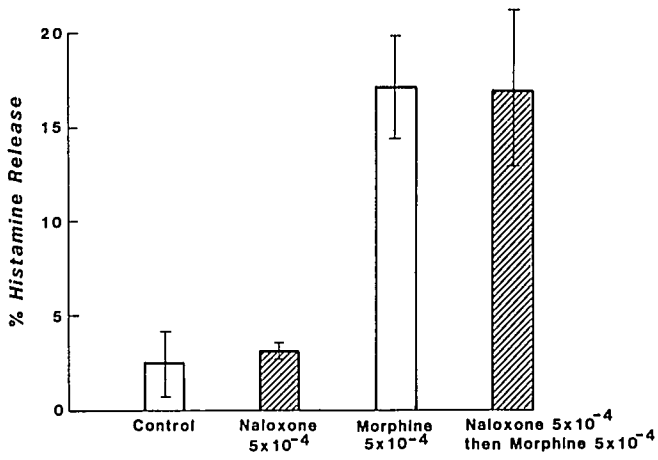


FIG. 3. Influence of preincubation with naloxone on morphine-induced histamine release from a human skin preparation. Naloxone alone produced no histamine release. Morphine produced similar histamine release in the presence and absence of naloxone.

### REPRODUCIBILITY OF DATA

In the same foreskin at the same drug concentration, the per cent histamine release varied by around 2%. In two separate foreskins, $1.5 \times 10^{-3}$ M morphine released 10.8% and 8.2% histamine in one and 16.5% and 18.1% histamine in the other.

In 15 different skins tested on either the same day or on different days, histamine release induced by $1.5 \times 10^{-3}$ M morphine varied from 8.2% to 22.7% histamine with a mean of 14.6% and a standard deviation of 3.7%. Because of this variability in histamine releasibility between foreskins, each skin was sectioned into 8–12 pieces and incubated with the whole range of drug concentrations in any one experiment.

## Discussion

Under the conditions employed in our experiments, morphine, in concentrations as high as $1.0 \times 10^{-2}$ M, failed to induce detectable histamine release from human basophils in a mixed leukocyte preparation. In contrast, morphine in concentrations as low as $1.5 \times 10^{-4}$ M induced significant histamine release from tissue mast cells in human foreskin. Oxymorphone and fentanyl, at all concentrations, failed to induce histamine release in this preparation, indicating that histamine release by narcotics does not parallel analgesic potency nor is it a nonspecific effect of high drug concentration.

We can only speculate as to the failure of human basophils to release histamine in the presence of large concentrations of morphine. It is doubtful that the number of basophils present was sufficiently different from the number of mast cells in the foreskin preparation to account for the lack of histamine release. Indeed, the total amount of histamine in each experimental preparation was similar, and leukocytes released histamine when challenged with Con A. More likely, basophils in the leukocyte preparation and mast cells in the skin preparations differ significantly in mechanisms of binding or degranulation after exposure to opiates, further demonstrating functional differences between histamine-containing cells from different sources within a given species.[10]

If histamine release occurs in mast cells at clinically used concentrations, it may be relevant to our practice of anesthesia. Morphine in concentrations of 0.1 mg/ml ($1.5 \times 10^{-4}$ M) induces urticarial wheal and flare reactions when injected intradermally. The urticaria is presumed a result of tissue mast cell histamine release, and hence intradermal morphine injection often is used as a positive skin test control in allergic skin testing. Development of urticaria along a vein into which morphine is administered is a common clinical observation. A frequently administered concentration of morphine is 10 mg/ml ($1.5 \times 10^{-2}$ M), which is 100-fold greater than the concentration producing histamine release *in vitro* ($1.5 \times 10^{-4}$ M). However, a demonstration of histamine release by morphine is not proof that all the adverse reactions attributed to the drug are indeed due to the release of histamine from mast cells.

Our *in vitro* results of narcotic-induced histamine release are also consistent with human *in vivo* measurements of plasma histamine concentrations after intravenous administration of various narcotics. Histamine levels

increased after intravenous administration of morphine but not fentanyl.[15,16]

This *in vitro* model avoids clinical variables such as preoperative medications, concurrently administered anesthetic agents, or surgical manipulation, which could potentiate or inhibit mast cell function, thereby changing the results obtained for a test drug. Moreover, this *in vitro* model allows study of the influence of variable concentrations of test drugs and antagonists and of factors controlling histamine release.

Factors that may control histamine release by narcotics include the displacement of histamine from binding sites because of some structural characteristic of the drug,[17] activation of a narcotic receptor, cytotoxicity, or a nonspecific effect of high drug concentration.

The opiate receptor is defined in part by the structure activity relationship to a series of agonists. For the agents used in this study, on classical opioid receptor bioassays, the relative analgesic potencies are as follows: fentanyl greater than oxymorphone greater than morphine. However, histamine release in our studies did not correlate with analgesic potency. Histamine release by morphine was very much greater than by oxymorphone and fentanyl, clearly demonstrating that histamine release does not parallel analgesic potency of opiates.

Histamine release could be a nonspecific effect of high concentrations of narcotic drugs. Because of the greater analgesic potency of oxymorphone and fentanyl compared with morphine, their initial concentrations in blood will be substantially less than that of morphine, which could explain their apparent lack of histamine release *in vivo*. However, since our *in vitro* studies demonstrated that even at millimolar concentrations fentanyl and oxymorphone did not release histamine, histamine release by narcotics is not a nonspecific effect of high drug concentrations.

MacIntosh and Paton[17] postulated that histamine is displaced from mast cells by drugs with chemically similar basic amine structures. Our studies, however, dispute a displacement reaction as the mechanism of morphine-induced histamine release. The structures of morphine and oxymorphone are very similar with minor changes at the C-6 and C-14 position and at one double bond, yet morphine but not oxymorphone released histamine and equimolar concentrations of both fentanyl and oxymorphone failed to release histamine in our mast cell preparation.

It is unlikely that histamine released by morphine resulted from drug-induced cell membrane damage (cytotoxicity), since LDH did not increase.

The failure of oxymorphine and fentanyl to induce histamine release, and the failure of naloxone to prevent histamine release by morphine, suggests that histamine release by morphine is not dependent on opiate receptor binding or activation. This result differs from that of Grosman,[18] who found that naloxone inhibited histamine release to morphine in rat peritoneal mast cells. However, the concentrations of naloxone used ($10^{-2}$ M) was much higher than that used clinically. It is conceivable that the concentration of morphine that we used ($5 \times 10^{-4}$ M) was having its maximal effect. Therefore, a shift in reactivity might not have been seen.

Calcium is required for morphine-induced histamine release, but release is calcium independent between 1 and 4 mM $CaCl_2$. Tharp found significant changes in IgE-mediated histamine release over the 1–4 mM calcium range in human foreskin mast cells. This variability in calcium dependence suggests important differences in mast cell triggering between immunologic and nonimmunologic challenge.

In summary, using human skin mast cells, we have demonstrated histamine release by morphine but not fentanyl or oxymorphone at the same concentrations. Our studies demonstrate that morphine-induced histamine release is not due to a displacement mechanism, is not due to cytotoxicity, and does not involve an opiate receptor. This *in vitro* human foreskin mast cell population is useful in studying the factors regulating opiate-induced histamine release.

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