Opioids differ in their capacity to cause release of histamine. The effects of increasing concentrations of three opioids (morphine, buprenorphine, and fenanyl) were studied on the release of preformed (histamine and tryptase) and de novo synthesized prostaglandin D2 (PGD2) and peptide-leukotriene C4 (LTC4) chemical mediators from human peripheral blood basophils and mast cells isolated from skin tissues or lung parenchyma. Basophils released <5% of their histamine content and did not synthesize significant amounts of LTC4 when incubated with any of the opioids. Mast cells showed markedly different responses to the three opioids. Morphine (10^-3-3 X 10^-4 M), in a concentration-dependent manner, induced histamine and tryptase release from skin but not from lung mast cells, up to a maximum of 18.2 ± 1.9% and 13.0 ± 4.1 μg/10^6 cells, respectively. Morphine did not induce de novo synthesis of PGD2 from skin mast cells. Buprenorphine (10^-5-10^-4 M), in a concentration-dependent manner, caused histamine and tryptase release from lung but not from skin mast cells, to a maximum of 47.6 ± 7.2% and 35.1 ± 13.6 μg/10^6 cells, respectively. Buprenorphine also induced de novo synthesis of PGD2 and LTC4 from lung mast cells. Fenanyl (10^-7-10^-6 M) did not induce histamine and tryptase release or the de novo synthesis of PGD2 or LTC4 from any mast cells. Histamine release caused by buprenorphine from lung mast cells was slow (½ = 11.2 ± 3.6 min) compared with that induced by morphine from skin mast cells (½ < 1 min, P < 0.001). The releasing activity of buprenorphine on lung mast cells was abolished by lowering the temperature of the incubation buffer to 22° C and 4° C. Buprenorphine’s capacity to induce histamine release from lung mast cells was partially dependent on extracellular Ca++ and was not reduced by metabolic impairment caused by preincubation (15-40 min) with 2-deoxy-d-glucose plus antimycin A. Thus, there appear to be functional differences between human basophils and mast cells and between mast cells from different anatomic sites in response to the three opioids tested. (Key words: Analgesics, opioid; buprenorphine; fenanyl; morphine; basophils; mast cells. Enzymes: tryptase. Medication: histamine. Leukotriene C4, Prostaglandin D2.)

The number of anaphylactoid reactions during general anesthesia is increasing.1,2 These have been attributed to histamine release from human mast cells and basophils3-5 on the basis of findings that several classes of drugs can cause histamine release in vivo6-7 and in vitro8-10 and that histamine induces most of the metabolic and hemodynamic alterations observed during allergic reactions.11-16 Although muscle relaxants are the drugs most frequently implicated in these reactions,17,18 opioids, such as morphine, are also potent histamine-releasing drugs19-21 Recently, techniques have become available to isolate mast cells from human skin tissues22 and lung parenchyma23 Human basophils and mast cells from different tissues appear to vary markedly in their functional responses to neuromuscular relaxants9 and general anesthetics10 and with respect to a parameter called “releasability” that governs the release of preformed (histamine and tryptase) and de novo synthesized mediators (prostaglandin D2 [PGD2] and peptide-leukotriene C4 [LTC4]).24-26 For instance, several neuromuscular relaxants and general anesthetics selectively induce only histamine release from mast cells, and the percentage of release varies significantly among different donors.9,10

Preliminary observations indicate that human basophils and mast cells isolated from different anatomic sites vary in their capacity to release histamine when challenged with morphine. This opioid induces histamine release from skin mast cells19,20 but not from basophils20 or from lung and heart tissue.21 However, the effects of morphine and other opioids on the release of tryptase, a useful marker of anaphylactoid reactions,27,28 and de novo synthesized mediators (LTC4 and PGD2) from human basophils and mast cells isolated from different anatomic sites have not been systematically evaluated.

This study was undertaken to compare the effect of morphine, a μ receptor agonist, with that of buprenorphine, a semisynthetic opioid acting as a partial agonist on the μ receptor, and with that of fenanyl, a synthetic μ agonist, on the release of histamine and tryptase and de novo synthesized chemical mediators (LTC4 and PGD2) from human basophils and mast cells isolated from skin tissues or lung parenchyma.

Materials and Methods

Reagents

The following were purchased: deoxyribonuclease, pronase, 2-deoxy-d-glucose, and compound A29187
(Calbiochem, La Jolla, CA); RPMI 1640 with 25 mM HEPES buffer and Eagle's minimum essential medium (Flow Laboratories, Irvine, Scotland); Hanks' balanced salt solution (HBSS) and fetal calf serum (Gibco, Grand Island, NY); $\text{H}_{2}\text{PO}_{4}$ (210 Ci/mmole) and $\text{H}_{2}\text{SO}_{4}$ (98.3 Ci/mmole) (New England Nuclear, Boston, MA); dextran T70 (Pharmacia LKB Biotechnology, Uppsala, Sweden); and piperoxone-N,N'-bis(2-ethane-sulfonic acid) (PIPES); elastase type I, hyaluronidase, chymopapain, collagenase, antimony A, and synthetic PGE$_2$ and LTC$_4$ (Sigma Chemical Co., St. Louis, MO). The following were kindly donated: morphine hydrochloride (Molteni & C., Florence, Italy), buprenorphine hydrochloride (Boehringer Biochemica Robini S.p.A., Milan, Italy), and fentanyl citrate (Farmitalia Carlo Erba, Milan, Italy). Rabbit anti-human Fcε (anti-IgE) and anti-PGE$_2$ and anti-LTC$_4$ antisera were generous gifts from Dr. T. Ishizaka and Dr. K. Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA) and Dr. L. M. Lichtenstein (The Johns Hopkins University Asthma and Allergy Center, Baltimore, MD), respectively. The tryptase radioimmunoassay kit (Pharmacia Tryptase RIACT 50, Pharmacia Diagnostics AB, Uppsala, Sweden) was kindly donated by Pharmacia S.p.A. (Cologno Monzese, Italy).

**Histamine Release from Human Basophils**

Informed consent was obtained from all subjects. Approximately 50 ml blood were drawn into a final concentration of 0.008 M ethylene diamine tetraacetate acid and 1.1% dextran 70. The erythrocytes were allowed to settle for 90 min at 22°C, and leukocyte-rich plasma was removed. The cells were separated by centrifugation at 180 × g for 8 min; washed twice in 25 mM PIPES (pH 7.35), 110 mM NaCl, 5 mM KCl; and resuspended in the same solution plus 1.0 mM CaCl$_2$ (PC): 0.4 ml samples of the cell suspension, containing 3 × 10$^4$ basophils per tube, were placed in Falcon 12 × 75-mm polyethylene tubes (Becton-Dickinson Labware, Lincoln Park, NJ) and warmed to 37°C. Morphine, buprenorphine, and fentanyl in powder form were dissolved in buffer immediately before each experiment. Each was diluted to final concentrations in the appropriate buffer and added to tubes in 0.2-ml aliquots, and incubation was continued at 37°C for 45 min. After centrifugation, the cell-free supernatant was assayed for histamine with an automated fluorometric technique.$^{31}$ The net percentage release was calculated by subtracting the histamine released spontaneously by the unstimulated samples from the total histamine released by cells lysed with 2% HClO$_4$.29 Mean spontaneous release of histamine in PC was 3.8 ± 0.9% of the available histamine content (mean ± SEM). The difference between replicate histamine measurements was < 10%.

**Isolation of Human Skin Mast Cells**

Skin was obtained from patients undergoing either mastectomy for breast cancer or elective cosmetic surgery procedures. General anesthesia in these patients was induced using the following drugs: thiopental, pancuronium, atropine, fentanyl (premedication); enflurane or isoflurane (anesthesia). Tissue was immediately placed in minimum essential medium at 4°C and used within 1 h. The skin was separated from the subcutaneous fat by blunt dissection. The remaining tissue was chopped into 1–2-mm fragments with scissors. The fragments were washed twice in calcium- and magnesium-free HBSS at 22°C and incubated for 3 h at 37°C with constant stirring in a solution of calcium- and magnesium-free HBSS containing 20 mg collagenase per gram wet weight of tissue, 5 mg hyaluronidase per gram wet weight of tissue, and 1,000 U/ml deoxyribonuclease.$^{33}$ The isolated cells were separated from the partially digested tissue fragments by filtration through Nitex cloth (Tetko, Elmford, NY; 150-μm pores) and stored. The remaining tissue was digested a second time with a fresh batch of the enzymes for an additional 2 h at 37°C. The cells were separated from the residual tissue fragments by filtration through Nitex cloth (as above), washed twice, combined with the cells from the first digestion, and washed again in calcium- and magnesium-free HBSS. Yields with this technique ranged between 1 × 10$^8$ and 9 × 10$^8$ mast cells, and purities were between 1% and 4%.

**Isolation of Human Lung Mast Cells**

Human lung tissue was obtained from patients undergoing thoracotomy and lung resection, mostly for lung cancer. General anesthesia in these patients was induced with the following drugs: droperidol plus fentanyl and atropine (premedication), and droperidol plus fentanyl, thiopental, succinylcholine, and pancuronium (anesthesia). Macroscopically normal lung tissue was dissected free from pleura, bronchi, and blood vessels; minced into 5–10-mm fragments; and dispersed into single-cell suspension as previously described.$^{23}$ Yields with this technique ranged between 3 × 10$^6$ and 18 × 10$^6$ mast cells, and purities ranged between 1% and 8%. Mast cells and basophils were stained with alcian blue and counted in a Spiers-Levy eosinophil counter.

**Mediator Release from Human Skin and Lung Mast Cells**

Aliquots of 0.4 ml of the cell suspension containing 3 × 10$^4$ mast cells per tube were placed in Falcon 12 × 75-mm polyethylene tubes and warmed to 30°C (skin mast cells) or 37°C (lung mast cells); 0.2 ml of each releasing stimulus was added, and incubation was continued...
at the same temperatures for 45 min. Experiments with
mast cells from skin of adult donors were performed at
30° C because adult skin mast cells release better at 30°
C than at 37° C. The remainder of the procedure and
the calculation of histamine release were identical to the
methods for basophils. In some experiments, 200-μl frac-
tions were taken from supernatants for the analysis of
tryptase, PGD₂, and LTC₄ concentrations. Mean sponta-
neous release of histamine in PC was 9.5 ± 1.0% of
cellular content in human skin mast cell preparations
and 8.9 ± 0.9% in human lung mast cell preparations.

**HISTAMINE ASSAY**

Histamine concentrations in supernatants from baso-
phil and skin or lung mast cell preparations were mea-
sured with the automated fluorometric method. At
the concentrations used, the three opioids did not interfere
with the fluorometric assay of histamine. The presence
of histamine in the samples was confirmed by degradation
with porcine histaminase.

**RADIOIMMUNOASSAY OF PROSTAGLANDIN D₂,
PEPTIDE-LEUKOTRIENE C₄, AND TRYPTASE**

PGD₂ and LTC₄ were evaluated by radioimmunoassay
as described previously. The rabbit anti-LTC₄ and
anti-PGD₂ antisera have been characterized and their
cross-reactivity for heterologous ligands described. Tryptase was analyzed by a solid-phase radioimmunoassay
(Pharmacia Tryptase RIAT 50); duplicate samples or
tryptase standards were added to plastic tubes coated with
mouse monoclonal anti-tryptase antibodies. Then ¹²⁵I-
radiolabeled anti-tryptase was added to the tubes, which
were incubated for 16-18 h at 22° C. After washing (3
× 2 ml saline, 22° C), bound radioactivity was counted
and gave a linear concentration-response curve within a
range of 2-50 μg/l.

**STATISTICAL ANALYSIS**

The results were expressed as the mean ± SEM. Static-
tistical significance was analyzed by two-way nonpara-
metric analysis of variance (Friedman X² test); multiple
comparisons were made by the extended Tukey test. Dif-
fences between matched pairs were analyzed by the
Wilcoxon signed-rank test. Correlations were calculated
by Spearman's nonparametric test.

**Results**

In a first series of experiments, we assessed the effects
of increasing concentrations of morphine, buprenorphine,
and fentanyl on mediator release from peripheral blood
basophils and mast cells isolated from skin tissues or lung
parenchyma. Morphine (10⁻⁵-3 × 10⁻⁴ M) and bupre-

t
norphone (10⁻⁶-10⁻⁴ M) did not induce histamine release
from basophils obtained from 10 and 11 donors, respecti-
vely (fig. 1). Tryptase was not measured because baso-
phil contained < 0.04 pg/cell tryptase, as previously re-
ported. In six experiments with human skin mast cells,
morphine induced concentration-dependent histamine
release, whereas in six experiments with lung mast cells
it did not (fig. 1). Morphine induced a maximum of 18.2
± 1.9% of histamine release from skin mast cells at the
highest concentration used (3 × 10⁻⁴ M).

Tryptase is a protease found in the granules of human
skin (~ 35 pg/cell) and lung (~ 10 pg/cell) mast cells. IgE- and
A23187-mediated activation of human skin mast cells
leads to release of tryptase, in addition to release of
histamine (table 1). Morphine also induced concentra-
tion-dependent tryptase release from skin mast cell prepara-
tions (table 1). There was a significant positive correlation
between the percentages of histamine and tryptase release
(r = 0.54; P < 0.05) from human skin mast cells.

Buprenorphine (10⁻⁶-10⁻⁴ M) did not induce histamine
release from skin mast cells from 15 donors, whereas in

![Fig. 1. Effect of increasing concentrations of morphine and buprenorphine on histamine release from basophils and from mast cells isolated from human skin (HSMC) and lung parenchyma (HLMC). The results are the mean ± SEM of the number of experiments indicated in the text. Error bars are not shown when graphically too small. *P < 0.01 compared to spontaneous release.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931324/)
15 experiments with lung mast cell preparations histamine release was concentration-dependent to a maximum of 47.6 ± 7.2% at 10^{-4} M. It is important to note that maximum histamine release caused by 10^{-4} M buprenorphine in these experiments ranged from 11.5% to 77.3%.

To assess the ability of lung mast cells to respond to IgE- and non-IgE-mediated stimuli, in each experiment cells were also challenged with an optimal concentration of anti-IgE (1 μg/ml) or Ca^{2+} ionophore A23187 (1 μg/ml). Anti-IgE and A23187 caused 24.5 ± 1.3% and 69.7 ± 2.2%, respectively, of histamine release from lung mast cells.

Buprenorphine (10^{-5}–10^{-4} M) induced concentration-dependent tryptase release from lung mast cell preparations (table 2). IgE-mediated activation of human lung mast cells also leads to release of tryptase, in addition to histamine (table 2). There was a significant positive correlation between the percentage of histamine and tryptase release induced by buprenorphine (r_s = 0.72; P < 0.05) or by anti-IgE (r_s = 0.83; P < 0.05) from human lung mast cells (fig. 2).

Fentanyl (10^{-3}–10^{-5} M) did not induce histamine release from basophils of 7 donors or from skin and lung mast cells from 12 and 7 donors, respectively (table 3). The highest concentration (10^{-5} M) of fentanyl induced > 5% of histamine release from skin mast cells from only 3 of 12 donors. Fentanyl (10^{-6}–10^{-3} M) also did not induce tryptase release from skin or lung mast cells (data not shown).

We next compared the kinetics of histamine release induced by buprenorphine and anti-IgE from lung mast cells, and by morphine and anti-IgE from skin mast cells. In five experiments the time course of histamine release induced by buprenorphine was slow, reaching a maximum 30–45 min after addition of the stimulus. Anti-IgE-induced histamine release was significantly faster, being complete after 10 min (fig. 3A). The kinetics of morphine- and anti-IgE–induced release in five experiments was also extremely rapid, reaching its plateau after 5 min (fig. 3B). The t/2 of the release reaction induced by buprenorphine in lung mast cells (11.2 ± 3.6 min) was significantly longer than for anti-IgE in lung mast cells (1.8 ± 0.1 min; P

---

**Table 1.** Effects of Morphine, Anti-IgE, and A2S187 on Histamine and Tryptase Release from Human Skin Mast Cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Net Histamine Release (%)</th>
<th>Tryptase Release (μg/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^{-4} M</td>
<td>10.8</td>
<td>18.3</td>
</tr>
<tr>
<td>3 × 10^{-4} M</td>
<td>13.7</td>
<td>21.6</td>
</tr>
<tr>
<td>Anti-IgE 1 μg/ml</td>
<td>22.5</td>
<td>12.7</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Morphine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^{-3} M</td>
<td>6.3</td>
<td>6.2</td>
</tr>
<tr>
<td>3 × 10^{-4} M</td>
<td>17.3</td>
<td>13.1</td>
</tr>
<tr>
<td>Anti-IgE 1 μg/ml</td>
<td>16.1</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Morphine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 × 10^{-4} M</td>
<td>17.7</td>
<td>4.4</td>
</tr>
<tr>
<td>A2S187 1 μg/ml</td>
<td>51.4</td>
<td>6.7</td>
</tr>
<tr>
<td><strong>Experiment 4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Morphine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^{-4} M</td>
<td>10.2</td>
<td>8.7</td>
</tr>
<tr>
<td>3 × 10^{-4} M</td>
<td>14.6</td>
<td>10.1</td>
</tr>
<tr>
<td>Anti-IgE 1 μg/ml</td>
<td>13.5</td>
<td>7.9</td>
</tr>
</tbody>
</table>

**Table 2.** Effects of Buprenorphine and Anti-IgE on Histamine and Tryptase Release from Human Lung Mast Cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Net Histamine Release (%)</th>
<th>Tryptase Release (μg/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Buprenorphine (M)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^{-5}</td>
<td>5.5</td>
<td>0.0</td>
</tr>
<tr>
<td>3 × 10^{-5}</td>
<td>7.3</td>
<td>0.2</td>
</tr>
<tr>
<td>10^{-4}</td>
<td>19.2</td>
<td>15.5</td>
</tr>
<tr>
<td>Anti-IgE 1 μg/ml</td>
<td>35.7</td>
<td>54.0</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Buprenorphine (M)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^{-5}</td>
<td>0.9</td>
<td>3.1</td>
</tr>
<tr>
<td>3 × 10^{-5}</td>
<td>1.2</td>
<td>6.6</td>
</tr>
<tr>
<td>10^{-4}</td>
<td>21.3</td>
<td>12.6</td>
</tr>
<tr>
<td>Anti-IgE 1 μg/ml</td>
<td>19.0</td>
<td>21.5</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Buprenorphine (M)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^{-3}</td>
<td>3.3</td>
<td>2.4</td>
</tr>
<tr>
<td>3 × 10^{-5}</td>
<td>3.3</td>
<td>8.8</td>
</tr>
<tr>
<td>10^{-4}</td>
<td>23.9</td>
<td>15.3</td>
</tr>
<tr>
<td>Anti-IgE 1 μg/ml</td>
<td>20.0</td>
<td>16.8</td>
</tr>
<tr>
<td><strong>Experiment 4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Buprenorphine (M)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^{-3}</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>3 × 10^{-5}</td>
<td>2.8</td>
<td>0.9</td>
</tr>
<tr>
<td>10^{-4}</td>
<td>76.0</td>
<td>32.4</td>
</tr>
<tr>
<td>Anti-IgE 1 μg/ml</td>
<td>11.3</td>
<td>9.5</td>
</tr>
</tbody>
</table>

**Fig. 2.** Correlation between the percent histamine release and tryptase secretion from human lung mast cells induced by buprenorphine (filled circles) or anti-IgE (open circles).
< 0.05) and for morphine in skin mast cells (<1 min; P < 0.001).

In five experiments, we investigated the mechanism of the histamine-releasing activity of buprenorphine (3.5 × 10⁻⁵ m) on lung mast cells at 37, 22, and 4°C. Buprenorphine’s releasing activity was abolished by lowering the temperature of the incubation buffer from 37 to 22 or 4°C (fig. 4).

FIG. 4. Effect of buprenorphine (3.5 × 10⁻⁵ m) on histamine release from human lung mast cells incubated 45 min at 37, 22, or 4°C. The results of five experiments are shown. Each bar corresponds to the mean of duplicate samples.

The effect of the presence of extracellular Ca²⁺ (1 mM) on the histamine-releasing activity of increasing concentrations of buprenorphine on lung mast cells is illustrated in figure 5. The activity was not abolished but was significantly less in the absence of extracellular Ca²⁺.

The biochemical events underlying the release of histamine caused by opioids from human mast cells are not known. We evaluated the effect of cellular metabolic impairment on buprenorphine-induced histamine release from lung mast cells. Preincubation of human lung mast cells for 20 min with 10 mM 2-deoxy-D-glucose and 1 μM antimycin A did not affect the release of histamine caused by buprenorphine (table 4). In the same experiments,

FIG. 5. Effects of various concentrations of buprenorphine on histamine release from human lung mast cells incubated 45 min at 37°C in the absence or presence of extracellular Ca²⁺ (1 mM). Mean ± SEM of five experiments. Error bars are not shown when graphically too small. *P < 0.05 compared to release in the absence of Ca²⁺.
TABLE 4. Effect of Metabolic Impairment on Buprenorphine-, Anti-IgE, and A23187-induced Histamine Release from Human Lung Mast Cells

<table>
<thead>
<tr>
<th></th>
<th>Net Histamine Release (%)</th>
<th>Inhibition of Histamine Release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>+ 2DG/ Antimycin A</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 × 10⁻⁸ M</td>
<td>12.4 ± 2.5</td>
<td>15.3 ± 2.9*</td>
</tr>
<tr>
<td>Anti-IgE 1 µg/ml</td>
<td>13.0 ± 2.0</td>
<td>5.9 ± 1.5*</td>
</tr>
<tr>
<td>A23187 0.5 µg/ml</td>
<td>18.8 ± 2.9</td>
<td>8.6 ± 2.6*</td>
</tr>
</tbody>
</table>

Results are presented as the mean ± SEM of three experiments. Mast cells were incubated 20 min at 37°C in the presence of buffer (control) or 10 mM 2-deoxy-d-glucose (2DG) plus 1 µM antimycin A. Then the cells were challenged with buprenorphine, anti-IgE, or compound A23187 at the concentrations indicated, and release proceeded for 45 min at 37°C.

* P < 0.05 compared to control.

However, preincubation with 2-deoxy-d-glucose plus antimycin A more than halved the release of histamine induced by anti-IgE and by the Ca²⁺ ionophore A23187. Similar results were obtained when cells were preincubated for different times (15–40 min) with 2-deoxy-d-glucose and antimycin A before the challenge with buprenorphine and anti-IgE (data not shown).

The activation of human basophils and lung mast cells, but not skin mast cells, leads to the de novo synthesis of LTC₄ through the 5-lipoxygenase pathway in addition to the secretion of preformed histamine. Both skin and lung mast cells, but not basophils, also synthesize PGD₂. We investigated the effects of various concentrations of morphine, buprenorphine, and fentanyl on the de novo synthesis of mediators from basophils and mast cells. In three experiments, morphine did not induce the synthesis of PGD₂ from skin mast cells or LTC₄ from lung mast cells (table 5). As controls, anti-IgE and compound A23187 stimulated synthesis of PGD₂ from skin and LTC₄ from lung mast cells. Fentanyl (10⁻⁸–10⁻⁷ M) also did not induce de novo synthesis of PGD₂ and LTC₄ from skin or lung mast cells (data not shown).

Different results were obtained with buprenorphine. As shown in figure 6, buprenorphine (3 × 10⁻⁵–10⁻⁴ M) induced the release of PGD₂ from lung mast cells in seven of eight preparations, to a maximum of 25.2 ± 7.7 ng/10⁶ cells. It also induced the de novo synthesis of LTC₄ from lung mast cells. In the same experiments, anti-IgE and A23187 caused the de novo synthesis of these mediators. The maximum PGD₂ and LTC₄ production induced by buprenorphine in these experiments ranged from 12.1 to 67.5 ng/10⁶ cells and from 0.0 to 91.7 ng/10⁶ cells, respectively, and was significantly lower than that induced by anti-IgE (PGD₂: 75.7 ± 20.7 ng/10⁶ cells, P < 0.05; LTC₄: 68.4 ± 22.2 ng/10⁶ cells; P < 0.05).

**Discussion**

Our results indicate that opioids can induce the release of preformed and de novo synthesized chemical mediators from human mast cells. There appear to be three levels of heterogeneity, with regard to 1) the releasing activity of different opioids; 2) the capacity of primary effector cells of allergic reactions in humans (e.g., basophil granulocytes and mast cells isolated from different anatomic sites) to respond to each opioid; and 3) the capacity of opioids to induce de novo synthesis and/or the release of preformed mediators.
Peripheral blood basophils are essentially unresponsive to a wide spectrum of concentrations of the opioids used in this study. This extends previous observations with morphine^{19-21} and our recent finding that basophils from normal donors are only minimally responsive to muscle relaxants^{9} and general anesthetics.^{16} However, the in vitro model of basophil histamine release could be useful to identify patients with specific IgE against a common epitope present in several opioids.^{44}

Lorenz and coworkers demonstrated that porcine mast cells isolated from different anatomic sites showed remarkably different patterns of sensitivity to various opioids.^{45} We now demonstrate that also mast cells from different human tissues are heterogeneous with regard to the releasing activity of different opioids. In particular, fentanyl did not activate mast cells isolated from lung parenchyma and skin tissue of adult donors. Fentanyl also did not release histamine from infant chopped foreskins.^{19}

These in vitro results agree with the clinical observation that fentanyl did not induce histamine release in vivo.^{3,46}

In addition, they extend previous observations of the functional heterogeneity of human mast cells isolated from different anatomic sites in response to various activating stimuli.^{29,41,47}

We have confirmed that morphine selectively induces only histamine release from skin mast cells.^{41} Therefore, the drug acts as an incomplete secretagogue on skin mast cells, as previously shown for muscle relaxants and general anesthetics on skin and lung mast cells.^{10}

The capacity of buprenorphine to induce the release of chemical mediators from human basophils and mast cells has not been systematically evaluated in previous in vitro studies. Our findings are that buprenorphine induced the concentration-dependent release of preformed mediators (histamine and tryptase) selectively from lung mast cells. Buprenorphine also induced the de novo synthesis of PGD₂ and LTC₄ from these cells. It is noteworthy that the releasing activity of this drug varied markedly in different cell preparations, as shown for muscle relaxants,^{9} suggesting that releasability itself^{24-26} influences the effect of buprenorphine on lung mast cells.

Awareness that buprenorphine and morphine can induce the secretion of tryptase, as well as histamine, might be useful for detection of anaphylactoid reactions to the two drugs. Once secreted, histamine is catabolized rapidly in human plasma,^{14} whereas the increase of tryptase concentrations induced by in vivo activation of mast cells has a half-life of ~ 2 h.^{27} Therefore, it might be clinically useful to measure plasma concentrations of tryptase after anaphylactoid reactions caused by opioids and possibly other drugs.^{28}

The finding that buprenorphine induced not only the release of preformed mediators but also the de novo synthesis of variable amounts of PGD₂ and LTC₄ from lung mast cells suggests that histamine H₁ and H₂ antagonists may be insufficient to block the in vivo effects of mediator release associated with buprenorphine anesthesia.

Because the biochemical pathways underlying the release of histamine from lung mast cells induced by buprenorphine are still not clear, we have attempted to shed some light on the characteristics of this release. Histamine release evoked by buprenorphine had a longer time course than release triggered by morphine in skin mast cells and by anti-IgE in lung and skin mast cells. Buprenorphine's releasing activity from lung and morphine's from skin mast cells was temperature-dependent because it was blocked in incubation buffer at low temperature (4°C). The effect of extracellular Ca²⁺ on morphine-induced histamine release from skin mast cells is still debated.^{19,47}

Our data indicate that buprenorphine-induced histamine secretion from lung mast cells was significantly lower in the absence of extracellular Ca²⁺.

We also investigated whether the histamine-releasing capacity of buprenorphine on lung mast cells was energy-dependent. Inhibition of glycolytic and oxidative metabolism within mast cells with the combination of 2-deoxy-D-glucose plus antimycin A diminished their response to anti-IgE and compound A23187, but buprenorphine's activity was not affected. The same combination of drugs blocked the histamine-releasing activity of morphine on skin mast cells.^{47} Taken together, these observations suggest that buprenorphine and morphine induce histamine release from lung and skin mast cells, respectively, through the activation of biochemical pathways that share some characteristics (e.g., temperature dependence) but not others (time course, mediators released, Ca²⁺ dependence, energy dependence).

Opioid compounds bind with multiple receptors also present on cells of the immune system, where they exert immunomodulatory effects.^{29,30} However, some of these effects are mediated by opioid receptor-independent mechanisms.^{19,47-50} Buprenorphine is a semisynthetic opioid closely related in structure to morphine,^{29,51} whereas fentanyl is a synthetic phenylpiperidine chemically not similar to morphine.^{29} Morphine, buprenorphine, and fentanyl all bind to µ receptors^{29,30} but have strikingly different activating properties on human mast cells. This suggests that their histamine-releasing activity is unlikely to be mediated by specific binding to opiate receptors. Additional studies with specific opioid receptor antagonists are necessary to characterize opioid receptors on human mast cells.

The in vitro drug concentrations inducing mediator release are higher than those usually found in the peripheral blood of patients undergoing general anesthesia with the drugs tested.^{52} However, the local concentrations of these drugs during or immediately after intravenous infusion might be relevant, considering the variability in the re-
leasing activity of cells from different donors and the differences in pharmacokinetics in different clinical conditions. Finally, although our experimental procedures included extensive washing of lung parenchyma and skin tissues and of the isolated mast cells, drugs used in premedication and for anesthesia may be partly responsible for the differences found in the responses of mast cells and basophils to opioids.

Previous studies with morphine, and general anesthetics using peripheral blood basophils and mast cells isolated from different anatomic sites have already shown that the releasing activity differs depending on the drug and the type of cells examined. The present results confirm that histamine release from peripheral blood basophils is not the ideal system for studying the releasing capacity of these drugs. General anesthetics selectively induce only histamine release from human mast cells. We now show that buprenorphine can also induce the de novo synthesis of chemical mediators from lung mast cells.

These findings have a number of implications. First, the mediator-releasing activity of new anesthetic drugs should be tested on mast cells isolated from different anatomic sites. Second, it might be useful to measure not only histamine release but also the release of other mediators, such as peptide-leukotrienes and PGD2, that exert profound hemodynamic effects, or tryptase, a marker of mast cell activation in vivo.

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