Wheat and Flare Responses to Opioids in Humans

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Certain opioids release histamine from cutaneous mast cells to produce local wheal and flare responses and adverse hemodynamic effects. In vivo responses to opioids suggest that cutaneous responses result from the interaction of opioids with opioid receptors on human mast cells. There are no data evaluating or comparing the opioids currently used in anesthesia. Volunteers were injected intradermally with different opioids as well as with naloxone and antihistamines to evaluate their effects on cutaneous mast cell reactivity and cutaneous vascular responses. Fentanyl and morphine produced concentration-dependent wheal and flare responses in the range of $5 \times 10^{-9}$ M to $1.5 \times 10^{-7}$ M. Volunteers were then tested intradermally with different opioids and histamine at a $5 \times 10^{-7}$ M concentration to determine their relative cutaneous effects. Morphine, meperidine, fentanyl, and sufentanil produced both wheal and flare responses that were significantly greater than those due to saline ($P < 0.05$). Naloxone, alfentanil, and nalbuphine did not produce significant wheal or flare responses. Butorphanol was followed by a significant wheal but no flare. Naloxone attenuated cutaneous wheal and flare responses to fentanyl and the flare response to morphine. Intradermal antihistamines (diphenhydramine and cimetidine) produced significant wheal and flare responses. Electron micrographs of biopsies from fentanyl-induced wheals demonstrated normal mast cell architecture with no evidence of mast cell degranulation. Opioid effects on wheal and flare responses and mast cell degranulation appear independent of opioid analgesic potency. Opioids produce cutaneous vascular responses dependent on both histamine release from mast cells and direct effects on the vasculature. Wheal and flare responses of opioids appear to represent an indicator of systemic histamine release only if they are of the same magnitude as that produced by an equimolar intradermal histamine injection. (Key words: Analgesics: opioids. Complications: opioid histamine release. Histamine: mast cells.)

OPIOIDS are part of a diverse group of molecular structures known to release histamine from human cutaneous mast cells.1–6 Histamine-mediated and direct vascular responses appear to represent mechanisms for hypotension during opioid anesthesia, especially with rapid intravenous injection. Morphine, when administered intravenously, releases histamine causing hypotension, flushing, and pruritis.5 Fentanyl is thought not to release histamine but it causes mild pruritus, flushing, and vasodilation. Although the relative histamine-releasing effects of different opioids at equianalgesic concentrations have been reported, comparisons have not been made at equimolar concentrations.7 Elucidating the structure and activity relationships of opioid-mediated histamine release and cutaneous vascular responses can facilitate the development of drugs devoid of histamine-releasing actions and potentially less likely to produce hemodynamic changes in large, anesthetic doses. Histamine release following intradermal injection of anesthetic drugs known to degranulate mast cells produces wheal and flare responses in humans.8 Histamine released by cutaneous mast cells after intradermal drug injection produces increased capillary permeability with palpable tissue edema, which is called a wheal response, as well as cutaneous vasodilation with local cutaneous redness, which is called the flare response.9 We evaluated the effects of different opioids and specific antagonists injected intradermally on mast cell reactivity and vascular responses by measuring wheal and flare responses.

Methods

SKIN TESTING

Following approval by the Human Investigations Committee and informed consent, healthy volunteers were given intradermal injections of different opioids, naloxone, and antihistamines as outlined in table 1. Dose–response curves were determined by injecting eight volunteers with $5 \times 10^{-6}$ M, $5 \times 10^{-5}$ M, $5 \times 10^{-4}$ M, and $1.5 \times 10^{-3}$ M concentrations of morphine sulfate and fentanyl citrate. Another 16 volunteers received intradermal injections of $5 \times 10^{-4}$ M solutions of morphine sulfate (Elkins-Sinn), meperidine hydrochloride (Winthrop-Breon), fentanyl citrate (Janssen), sufentanil citrate (Jansen), alfentanil hydrochloride (Janssen), nalbuphine hydrochloride (Du Pont), butorphanol tartrate (Bristol), and naloxone hydrochloride (Du Pont).

An additional 12 volunteers received intradermal injections of morphine sulfate, fentanyl citrate, naloxone hydrochloride (N), diphenhydramine hydrochloride and cimetidine hydrochloride (D+C), morphine sulfate + N, morphine sulfate + D+C, fentanyl citrate + N, and fentanyl citrate + D+C, all drugs at a final concentration of $5 \times 10^{-4}$ M in their injected form. Drugs were preservative-free except the nalbuphine solution, which contained sodium metabisulfite, methylparaben, and propyl-

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paraben. Fentanyl and sufentanil solutions were prepared from the crystallized drug solubilized in sterile water. All skin testing was accompanied by negative (normal saline) and positive (histamine phosphate at $5 \times 10^{-4}$ M) controls. Drugs in a total volume of 0.02 ml were injected intradermally through a 26-g needle along the anterior aspect of the left and right forearms. Solutions were freshly prepared and all drugs were tested simultaneously in each volunteer. The largest diameters of wheal (induration) and flare (redness) were measured in millimeters for each drug injection 15 min after the injections. The individual measuring wheal and flare response was not blinded to the solutions. Data were compared by one-way analysis of variance and a Student-Neuman-Keuls test for multiple comparison, and $P < 0.05$ was considered statistically significant.

**SKIN BIOPSIES**

Fentanyl (0.02 ml of $5 \times 10^{-4}$ M solution) was injected intradermally into one forearm of three different human volunteers. After peripheral four-quadrant injections of 1% lidocaine, a 2-mm punch biopsy specimen was taken from the wheal induced by fentanyl 10 min after injection. The biopsy specimen was immersed in Karnofsky’s fixative and processed for transmission electron microscopy. After processing semithin sections were cut and stained with toluidine blue. They were examined by light microscopy to select areas with mast cells for thin sectioning. Thin sections (700 nm) were then cut, counterstained with lead citrate and uranyl acetate, and examined in a Phillips 201® electron microscope.

**Results**

**DOSE-RESPONSES TO INTRADERMAL OPIOIDS**

Morphine produced concentration-related increases in both wheal and flare responses, all of which were significantly different from saline controls (fig. 1). Fentanyl-induced wheal and flare responses were significant only at $5 \times 10^{-4}$ M concentrations or greater.

**EQUIMOLAR OPIOID TESTING**

Sixteen volunteers were tested intradermally with $5 \times 10^{-4}$ M concentrations of morphine, meperidine, fentanyl, alfentanil, nalbuphine, butorphanol, naloxone, and histamine, and 13 of 16 volunteers received sufentanil. Morphine, meperidine, fentanyl, sufentanil, butorphanol, and histamine produced significant wheal responses ($P < 0.05$) (fig. 2). There were no significant differences in the wheal responses produced by morphine or meperidine.

**Table 1. Protocol for Intradermal Opioid Testing in Volunteers**

<table>
<thead>
<tr>
<th>Volunteers</th>
<th>Drugs Evaluated</th>
<th>Molar Concentrations Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Morphine sulfate</td>
<td>$5 \times 10^{-6}, 5 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>Fentanyl citrate</td>
<td>$5 \times 10^{-6}, 1.5 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>Normal saline</td>
<td>$5 \times 10^{-4}, 1.5 \times 10^{-3}$</td>
</tr>
<tr>
<td>16</td>
<td>Morphine sulfate</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Meperidine hydrochloride</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Fentanyl citrate</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Sufentanil citrate</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Alfentanil hydrochloride</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Nalbuphine hydrochloride</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Butorphanol tartrate</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Naloxone hydrochloride</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Histamine phosphate</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Normal saline</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>Naloxone hydrochloride (N)</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Diphenhydramine hydrochloride + cimetidine hydrochloride (D+C)</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Morphine sulfate</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Morphine sulfate + N</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Morphine sulphate + D+C</td>
<td>$5 \times 10^{-4}$</td>
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<tr>
<td></td>
<td>Fentanyl citrate</td>
<td>$5 \times 10^{-4}$</td>
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<tr>
<td></td>
<td>Fentanyl citrate + N</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Fentanyl citrate + D+C</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Histamine phosphate</td>
<td>$5 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>Normal saline</td>
<td>—</td>
</tr>
</tbody>
</table>

**Fig. 1.** Comparison of wheal and flare sizes to increasing concentrations of fentanyl and morphine. The values in parentheses indicate the number of different volunteers tested. Data are expressed as mean ± SD. Morphine produced dose-related increases in wheal and flare size versus saline controls at all concentrations, whereas fentanyl produced significant responses at ≥$5 \times 10^{-4}$ M. *P < 0.05 compared with saline controls.
SKIN BIOPSIES

Numerous mast cells were observed by electron microscopy of skin biopsied from fentanyl-induced wheals in three different volunteers. The mast cell architecture was normal (fig. 4). Granules within the mast cells displayed consistency in appearance, normal density, and structural organization and were intact. There was no evidence of mast cell degranulation.

Discussion

The ability of certain opioids to release histamine by mast cell degranulation is a well-documented phenomenon. Histamine released by cutaneous mast cells produces increased capillary permeability (wheal) and cutaneous

OPIOIDS WITH NALOXONE AND ANTIHISTAMINES

Twelve volunteers were tested intradermally with 5 × 10⁻⁴ M concentrations of naloxone (N), diphenhydramine and cimetidine (D+C), morphine, morphine + N, morphine + D+C, fentanyl, fentanyl + N, fentanyl + D+C, histamine, and normal saline. Diphenhydramine and cimetidine produced significant wheal and flare responses compared with saline controls (P < 0.05) and partially antagonized morphine, but not fentanyl-induced wheal and flare responses (fig. 3). Naloxone antagonized wheal and flare responses to fentanyl and reduced the flare response to morphine (P < 0.05).

**Fig. 2.** Wheal and flare size following intradermal injections of 5 × 10⁻⁴ M concentrations of opioids, histamine, and normal saline in 16 volunteers. Data are expressed as mean ± SD. *P < 0.05 compared with saline controls.

**Fig. 3.** Wheal and flare size following intradermal injection of normal saline, naloxone (N), diphenhydramine and cimetidine (D+C), morphine alone and with antagonists, fentanyl alone and with antagonists, and histamine in 12 volunteers. All drug concentrations are 5 × 10⁻⁴ M in their injected form. Data are expressed as mean ± SD. D+C produced significant wheal and flare responses compared with saline and attenuated the wheal and flare responses to morphine (P < 0.05). N attenuated the wheal and flare responses to fentanyl and the flare response to morphine (P < 0.05).
vasodilatation (flare). Prior studies evaluating histamine release by opioids in whole slices of human skin did not evaluate in vivo cutaneous vascular reactivity. The analgesic potencies of opioids differ, but few quantitative studies have been performed to assess the relative ability of different opioids to degranulate mast cells. Changes in plasma histamine concentrations following intravenous opioid administration may be insensitive indicators of histamine release because histamine is rapidly metabolized by endothelial cells. Intradermal drug administration provides a useful system to evaluate the effects of different opioids on vascular responsiveness and endogenous mast cell degranulation in humans.

We demonstrated significant wheal and flare responses following morphine and meperidine as well as fentanyl and sufentanil, but not alfentanil, at a concentration of $5 \times 10^{-4}$ M. This concentration was chosen on the basis of our dose–response data and prior studies demonstrating a plateau effect on histamine release at concentrations greater than $5 \times 10^{-4}$ M. Similar wheal responses by meperidine, morphine, and histamine suggest that equimolar concentrations of morphine and meperidine may displace equivalent amounts of histamine from cutaneous mast cells. We also noted the ability of fentanyl and sufentanil, but not alfentanil, to produce a wheal and flare. Prior studies have failed to demonstrate histamine release by either fentanyl or sufentanil.

Morphine, meperidine, fentanyl, sufentanil, and alfentanil showed variable effects in their ability to produce wheal and flare responses unrelated to their relative mu receptor order of potency: sufentanil > fentanyl > alfentanil > butorphanol > nalbuphine ≥ morphine > meperidine. A spectrum of pharmacologically unrelated compounds has the ability to produce human cutaneous mast cell degranulation. Therefore, it is not surprising that opioids exhibit variable effects on wheal and flare responses independent of mu receptor potency. This is further supported by the lack of cutaneous responses by alfentanil. Although Hermens et al. were unable to demonstrate histamine release in isolated cutaneous mast cells, we noted the ability of fentanyl and sufentanil to produce in vivo wheal and flare responses. These findings may be due to direct opioid-mediated capillary vasodilation because naloxone antagonized fentanyl-induced cutaneous drug responses. Although intradermal antihistamines partially antagonized the wheal and flare responses to
morphine, the antihistamines produced significant wheal
and flare responses of their own; this precludes any
interpretation of their interactions with fentanyl. However,
mast cell degranulation was not evident in skin biopsies
from wheal sites produced by fentanyl. This does not pre-
clude the possibility that nonmast cell histamine release
occurred from the vessel wall. However, antagonism of
fentanyl wheal and flare responses by naloxone and partial
antagonism of morphine-induced flare by naloxone sug-
gests direct opioid-mediated capillary vasodilatation as an
important mechanism in producing cutaneous responses.
Naloxone was more effective at decreasing the size of the
wheal and flare response to fentanyl because morphine’s
effect is related to both histamine release and direct opioid
effects, whereas fentanyl-induced effects appeared to be
related only to direct vascular responses. Experiments in
dogs have demonstrated that sufentanil produced neu-
rogenic vasodilation that was antagonized by α1-adreno-
ceptor blockade.14

In conclusion, intradermal injection of equimolar con-
centrations of opioids demonstrated variable abilities of
specific agents to produce wheal and flare responses in
humans. The intensity of these responses was not related
to the potency of the opioids as mu receptor agonists.
Opioids produced cutaneous vascular responses depend-
ent on both histamine release from mast cells and direct
effects of opioids on the vasculature. Attempts to antag-
onize the adverse hemodynamic effects of histamine-re-
leasing opioids with antihistamines will not be completely
effective due to direct vascular responses. Wheal and flare
responses by intradermal opioids may only be an indicator
of systemic histamine release if they are of the same mag-
nitude as that produced by an equimolar intradermal his-
tamine injection, suggesting that mast cell degranulation
by opioids is an all or nothing biologic response. Addi-
tional research in this area is necessary to elucidate me-
chanisms responsible for drug-induced mast cell degranu-
lation and cutaneous vascular responses.

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