Opioid-Induced Mast Cell Activation and Vascular Responses Is Not Mediated by μ-Opioid Receptors: An *In Vivo* Microdialysis Study in Human Skin

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Activation of mast cells and the systemic release of histamine is a common side effect of opioids. Nevertheless, fentanyl and its derivatives show only a slight activation of mast cells with a subsequent liberation of histamine and tryptase. In this study, we used intradermal microdialysis to assess whether this stimulatory effect of opioids on mast cells depends on the activation of opioid receptors. This new approach allowed us to measure the dose-dependent release of histamine and tryptase from mast cells and the subsequent vascular and sensory effect without systemic side effects in volunteers. The opiate codeine and the synthetic opioids meperidine, fentanyl, alfentanil, sufentanil, remifentanil, buprenorphine, and the opioid antagonist naloxone were tested. Only codeine and meperidine induced mast cell activation with the release of tryptase and histamine, leading to protein extravasation, flare reactions, and itch sensations. Because naloxone did not attenuate these effects, it is unlikely that μ-opioid receptors are involved in the activation of mast cells.

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The manifestation of an allergic reaction ranges from urticaria and rash to severe bronchoconstriction, laryngeal edema, hematological disorders, and other serious maladies. Many drugs, especially penicillins, β-lactam antibiotics, and sulfonamides, given in the perioperative setting, induce allergic reactions (1). Although true allergic reactions to opioids are rare, naturally occurring compounds such as morphine and codeine can cause allergic reactions and can even lead to anaphylactic shock (2,3), whereas reactions to semisynthetic and synthetic compounds are seldom seen. Opioid-receptor agonists and antagonists have been tested on their ability to induce mast cell degranulation in the skin (4,5). In *in vitro* settings, morphine liberated histamine and tryptase from mast cells isolated from skin (HSMC), whereas buprenorphine did so only from mast cells isolated from lung parenchyma (HLMC), and fentanyl was not able to liberate mediators from any kind of mast cell (6). Severe itching and reddening of the skin, especially around the site of IM or subcutaneous injections of morphine, have been documented. Mast cell degranulation and the consecutive release of vasoactive mediators such as histamine are regarded as the main mechanisms (7). After the epidural injection of morphine, generalized or segmental itch sensations occur that can be attenuated by histamine antagonists, e.g., promethazine (8). Whether the degranulation of cutaneous mast cells by opioids depends on an agonistic effect on opioid μ receptors is still not clear. Experiments with opioid receptor antagonists (naloxone), morphine, meperidine, and codeine suggest that both opioid and non-opioid receptors may be involved (9).

In the present study, using the technique of intradermal linear microdialysis, we assessed the ability of opioids to induce a dose-dependent degranulation of cutaneous mast cells and simultaneously measure the dermal effects of the mediators released from these mast cells. *In vivo* settings, in which intracutaneous injections of the respective drug have been made, offer

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only an indirect measurement of the degranulatory effects of mast cells, that is, the extent of the wheal and erythema formation. Using cutaneous microdialysis, the measurement of the released mediators and their vascular effects were assessed simultaneously *in vivo* (10). Besides using the potent mast cell degranulator codeine and morphine, a variety of semi-synthetic and synthetic opioid agonists and antagonists were investigated (meperidine, buprenorphine, fentanyl, alfentanil, sufentanil, remifentanil, and naloxone) to measure dose-response curves for protein extravasation, as well as for histamine and tryptase release. Moreover, laser Doppler imaging techniques with high spatial resolution were used to measure changes of local blood flow and blood flow changes arising from the induction of an axon reflex erythema.

Methods

Sixty healthy subjects (31 women and 29 men; mean age, 29.5 ± 5.5 yr; range, 21–40 yr) participated in this randomized, double-blinded study. They were assigned to 1 of 8 study groups, each receiving one opioid or opioid-receptor antagonist in five different concentrations. None had previously suffered from hypersensitivity to drugs, especially drugs used during anesthesiological procedures, or had taken medications that interfere with itch or pain sensations and flare response (i.e., analgesics, antihistamines, cromoglycate, calcium, or sodium channel blockers). Each subject gave written informed consent to take part in the study; the experimental protocol was approved by the Ethics Committee of the Medical Faculty of the University of Erlangen-Nuremberg.

The following drugs were used as stock solutions: Codeine phosphate (1 mg/mL; 3.34 mM), morphine hydrochloride (1 mg/mL; 3.11 mM, Merck, Whitehouse Station, NJ), meperidine hydrochloride (10 mg/mL; 40.4 mM, Dolantin®, Aventis, Bridgewater, NJ), buprenorphine (0.3 mg/mL; 0.64 mM, Temgesic®, Gruenenthal, Mitlodi, Switzerland), fentanyl (0.05 mg/mL; 0.15 mM, Bbraun, Sheffield, United Kingdom), alfentanil (0.5 mg/mL; 1.2 mM, Rapifen®, Janssen-Cilag, Neuss, Germany), sufentanil (5 μg/mL; 0.12 mM, Sufenta®, Janssen-Cilag), remifentanil (1 mg/mL; 2.65 mM, Ultiva®, Glaxo-Wellcome, Munich, Germany), and the opioid receptor antagonist naloxone (0.4 mg/mL; 1.22 mM, Narcanti®). Solutions of codeine, morphine, and meperidine were free of preservatives or other inactive components.

Intradermal microdialysis was used to administer the drugs intracutaneously without further injuring the skin. Five hollow plasmapheresis fibers with a diameter of 400 μm and a cutoff range of 3000 kD (Dermal Dialysis, Erlangen, Germany) were placed intracutaneously within the skin of the volar forearm using 25-gauge canula attached to the plasmapheresis fibers. Insertion length was approximately 15 mm, and the distance between each fiber was at least 30 mm oriented transversally to the axis of the forearm. The hollow fibers were perfused with Ringer's solution (Ringerlösung, Bad Homburg, Germany) using a microdialysis pump (Pump 22, Harvard Apparatus, South Natick, MA) at a flow rate of 4 μL/min. Tygon® tubes (Cole-Parmer Instrument Company, Vernon Hills, IL) were used to connect the hollow fibers with the syringes (inner diameter, 0.38 mm). Insertion depth was controlled by ultrasound (Dermascan C, Cortex Technologies, Denmark); the average depth ranging between 0.5 and 0.9 mm. The dialysate was collected using glass capillaries tilted at an angle of 5 degrees to minimize outflow resistance. Dialysate was sampled every 15 min for 120 min, and frozen at −20°C in polyethylene cups for further analysis. After a baseline of 60 min, each of the five fibers was perfused with different concentrations of one opioid receptor agonist or antagonist for 30 min. This stimulation period was followed by a 30-min washout period. After waiting for the skin to adapt to the insertion trauma, the administration of the drugs involved no further injury of the skin, because the delivery of the drug was driven by diffusion from the dialysis fibers into the surrounding tissue.

Each dialysate sample (60 μL) was analyzed for total protein (5 μL) and tryptase (30 μL) content. Protein was measured photometrically (MRX reader, Dynatech, Denkendorf, Germany) using Coomassie blue dye for the analysis, with bovine serum albumin as a standard. Tryptase was analyzed by fluorescent immunoassay (UniCAP 100, Pharmacia & Upjohn, Freiburg, Germany). Histamine was measured using a fiber-based spectrofluorometric assay (11).

Superficial blood flow of the forearm was measured at regular intervals by laser-Doppler imager (LDI, Moor Instruments Ltd, Devon, United Kingdom), scanning the injection sites within an area of 20 × 10 cm with a resolution of 16,380 pixels. Local vasodilation as well as the axon reflex vasodilation was measured every 15 min during the equilibration period and every 5 min during the stimulation period. During the washout period of 30 min, the scanning interval was changed back to 15 min. Images were processed by dedicated software (MoorLDI Version 3.0, Moor Instruments Ltd). Local vasodilation was determined within a 0.4-cm narrow and 1.5-cm long region of interest placed directly above the membranes. The mean flux of developing axon reflex vasodilation was determined by moving the region of interest previously placed above the membrane site exactly 1 cm proximally.

During the first minute after onset of stimulation, the subjects were asked to rate the maximum itch or pain sensation on a numeric rating scale (NRS) separately for each stimulation site. The end-points were defined as "no itch/pain" (NRS = 0) and "maximum itch/pain" (NRS = 10).

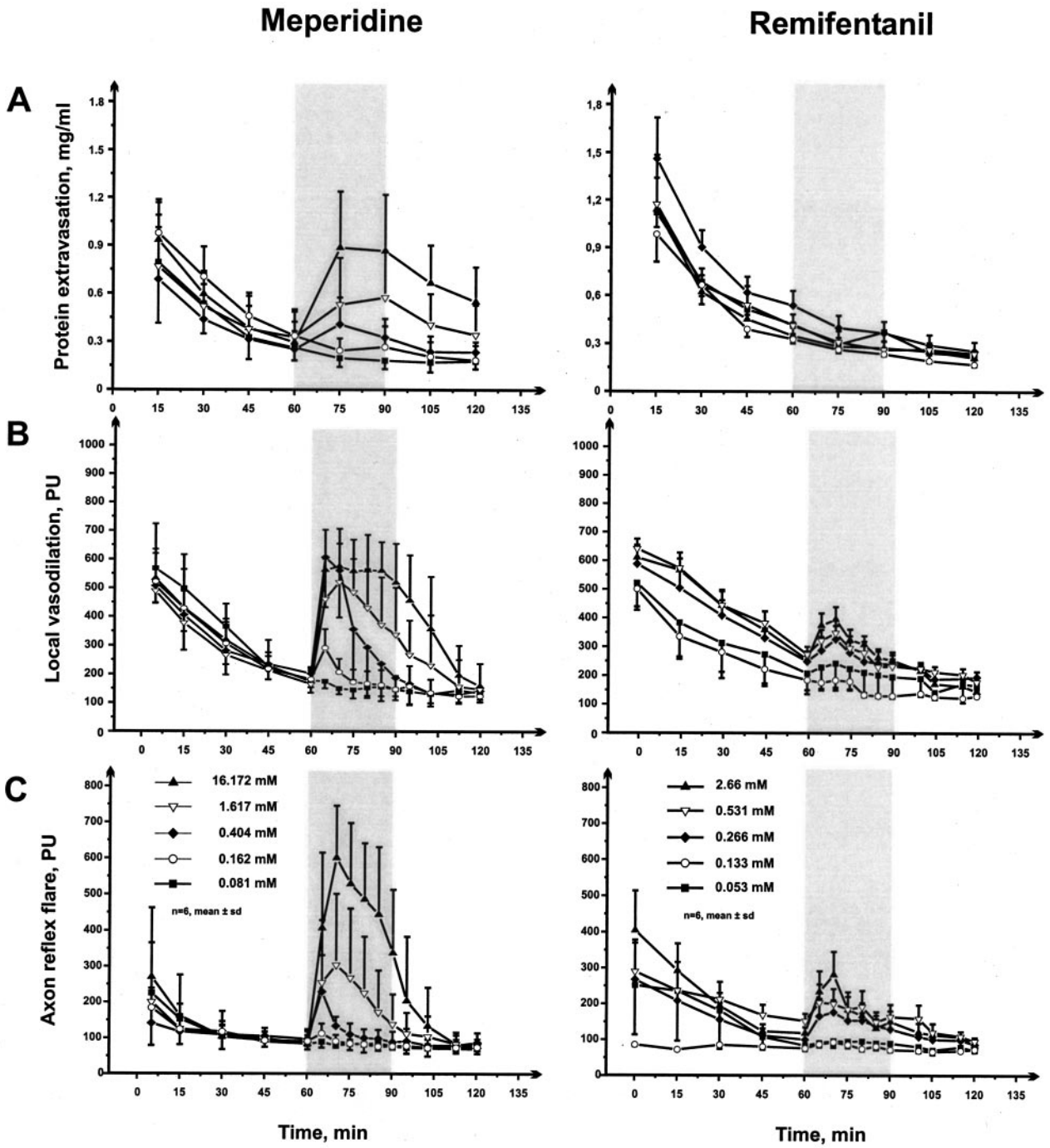

Figure 1. Time course of protein extravasation (A), local vasodilation (B), and axon-reflex erythema (C) after intradermal stimulation with five different concentrations of the opioid agonists meperidine and remifentanil ($n = 6$; mean ± SD). The significance level shown is $P < 0.01$. During the first 60 min, the intradermal compartment was perfused with Ringer's solution to enable the skin to compensate for the effects induced by the insertion trauma of the syringes. During the 30-min stimulation period, highlighted by the gray bar, meperidine induced a pronounced increase of protein extravasation, local vasodilation, and of axon-reflex erythema, whereas remifentanil was unable to cause protein extravasation. Only a slight, dose-independent increase of local and axon reflex blood flow was observed.

To assess whether the μ receptor antagonist naloxone is able to antagonize the effect of morphine (0.5 mg/mL), an agonist with a high-affinity to the μ receptor, of codeine (0.5 mg/mL), the prodrug to morphine with a low μ affinity, and of meperidine (1 mg/mL), showing low affinity to the μ receptor but high affinity to the κ-receptor, we co-injected these drugs with naloxone (0.2 mg/mL) intradermally. Blood flow changes were assessed by laser Doppler imaging, and the extent of the developing wheal reaction was measured 20 min after

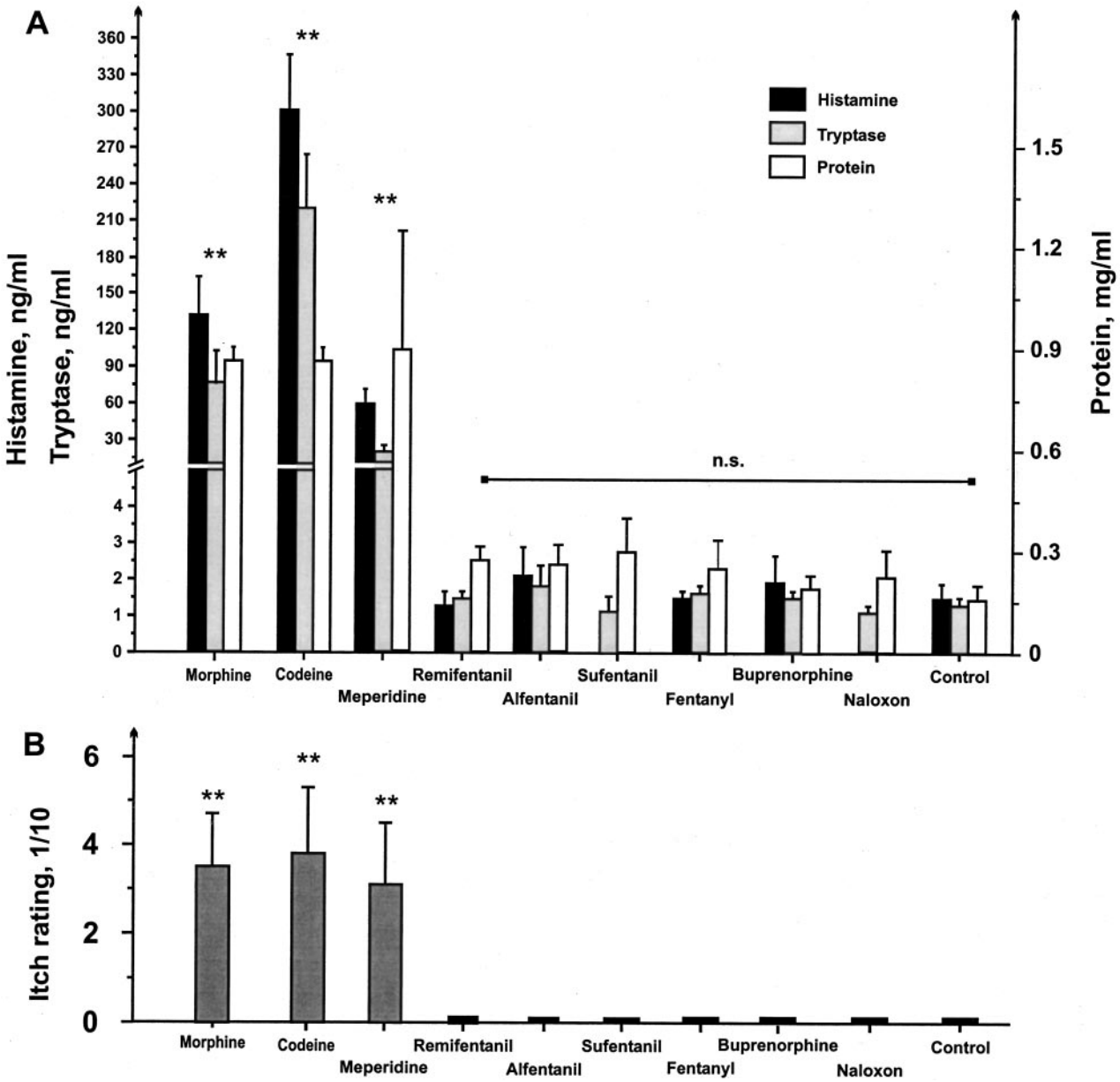

Figure 2. The top column graph depicts the amount of protein within the skin tissue and the amount of the mediators histamine and tryptase liberated from mast cells by the largest concentration of the respective drug. The bottom panel (B) shows the corresponding peak itch reaction, as rated by the subjects. For statistical analysis, a Friedman analysis of variance Wilcoxon matched pairs test, *post hoc*, was performed ($n = 6$, mean ± SD). The significance level shown is $P < 0.01$. Note that only the opioid agonists with a low affinity to the μ receptor meperidine, codeine, and morphine were able to induce a significant increase of histamine, tryptase, and protein extravasation, corresponding well with the itch sensation experienced by the subjects.

the injection. Moreover, the subjects were asked to rate the intensity of itch sensations using the NRS described above.

The *in vivo* delivery of fentanyl and buprenorphine was determined from the remaining dialysate. The delivery was calculated as the relative difference between the amount of opioid in the perfusate and the dialysate (percent decline in concentration) (12). Fentanyl and buprenorphine were analyzed using liquid chromatography mass-spectrometry (13).

Results are expressed as mean ± SD. Dose response curves for local vasodilation and for the axon reflex erythema represent the relative increase from baseline values during the entire stimulation period (six data points). These six data points were calculated as area under the curve. In some cases, this leads to negative values reflecting a further decrease during perfusion with the smallest concentrations. Potencies are given as concentration required for 50% of the maximum effect (ED_{50}). For each subject, ED_{50}s and effectiveness

(maximum effect) of the respective opioid were obtained by nonlinear regression fitting to the logistic function $y = y_{min} + (y_{max}\text{-}y_{min})/(1 + 10(\log x_0\text{-}x)*p)$, in which p is the Hill coefficient, y_{max} represents efficacy, and x_0 the concentration required for the ED_{50}. Origin software (Microcal, Northampton, MA) was used for these calculations. Data were compared using one-way analysis of variance followed by Newman-Keuls *post hoc* tests. Significance levels were $P < 0.05$. The STATISTICA software package (Statsoft, Tulsa, OK) was used for statistical analysis.

Results

During stimulation, *in vivo* delivery rates ranged between 25% and 35% for fentanyl and for buprenorphine ($n = 4$ each). Systemic effects such as miosis or respiratory depression were not observed. The insertion of the dialysis membranes induced a sharp burning pain with the subsequent formation of an axon-reflex erythema. The axon-reflex erythema completely disappeared after 60 min of baseline perfusion.

Intradermal stimulation with morphine, codeine, and meperidine led to a massive dose-dependant increase of protein extravasation and vasodilatation (Fig. 1) resulting from mast cell activation with tryptase and histamine release (Fig. 2). In addition, this liberation of histamine provoked distinct itch sensations. Stimulation with remifentanil, fentanyl, sufentanil, alfentanil, buprenorphine, and naloxone did not induce histamine or tryptase release.

Figure 1 depicts the time courses for protein extravasation, local vasodilation, and axon-reflex erythema formation. The highly potent μ receptor agonist remifentanil (100-fold potency compared with morphine) neither induced protein extravasation from dilated vessels, nor was it able to liberate tryptase or histamine from skin mast cells. Itch sensations after the administration of remifentanil, fentanyl, alfentanil, sufentanil, buprenorphine, or naloxone were not described by any of the subjects (Fig. 2, bottom panel). Local vasodilation and the development of an axon-reflex erythema occurred unspecific for all tested remifentanil concentrations (Fig. 1), showing only a slight linear increase (Fig. 3). The same time course was elicited by the other μ receptor agonists alfentanil, remifentanil, and sufentanil, with no protein extravasation and only unspecific vasodilatory effects. The partial μ receptor agonist buprenorphine and the antagonist naloxone did not induce protein extravasation or mediator liberation from cutaneous mast cells. The agonists with low μ receptor affinity, such as meperidine and codeine, and the high-affinity μ-receptor agonist morphine all were able to induce protein extravasation with wheal formation, intense local vasodilation, and equally intense axon-reflex flare development persisting over the 30-min stimulation period in the case of the largest concentrations administered.

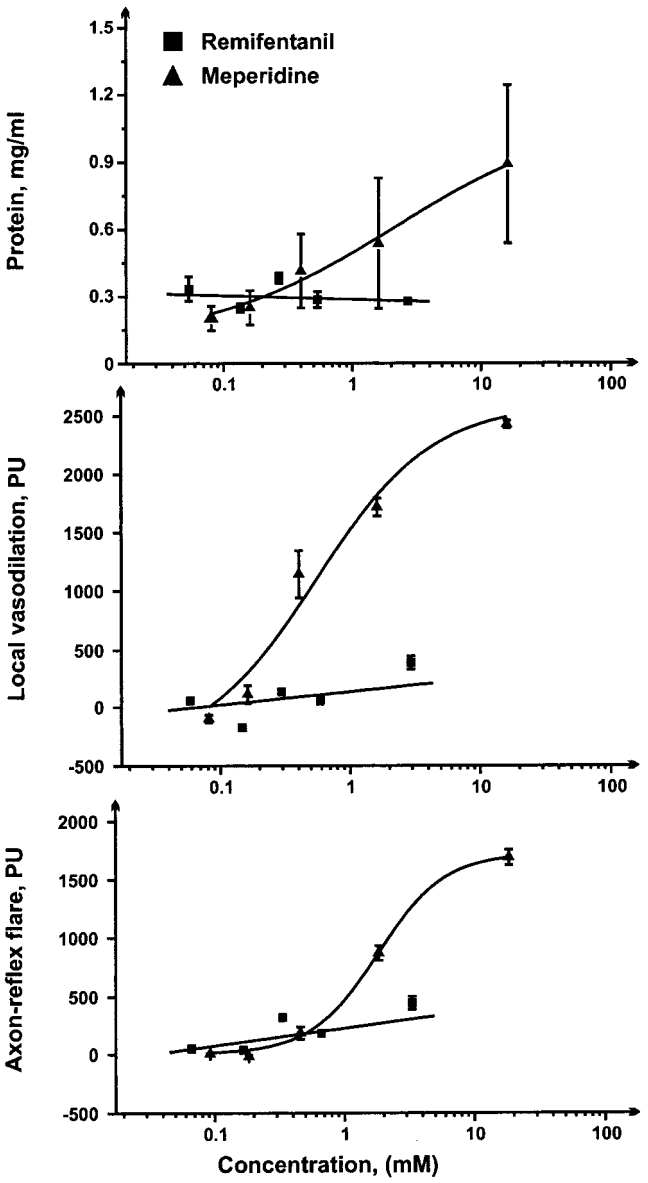

Figure 3. Dose-response curves of protein extravasation, local vasodilation, and axon-reflex erythema formation ($n = 6$; mean ± SD). Comparison of the low-affinity μ receptor agonist meperidine with the potent agonist remifentanil. Only meperidine showed dose-response activity, whereas remifentanil showed only an insignificant increase in all three variables.

As depicted in Figure 3, meperidine showed a dose-dependent increase in protein extravasation (efficacy, 0.93 ± 0,04; ED_{50} = 1.15 ± 0.7; Hill-coefficient = 0.88), local vasodilation (efficacy, 2504.5 ± 95.8; ED_{50} = 0.27 ± 0.29; Hill-coefficient = 0.82), and axon-reflex erythema (efficacy, 1714.6 ± 21.9; ED_{50} = 1.54 ± 0.09; Hill-coefficient = 1.57). However, remifentanil showed, as did all other potent opioids, no dose-dependent protein extravasation ($r = -0.28$), only a very slight linear increase of local vasodilation ($r = 0.38$), and an equally slight linear increase of the axon-reflex erythema ($r = 0.67$). Note that in contrast to the largest administered

Table 1. Naloxone (0.2 mg/mL) Combined with Morphine (0.5 mg/mL), Codeine (0.5 mg/mL), or Meperidine (1 mg/mL) does not influence the Intensity of the Developing Erythema, Flare Size, or Wheal Development

	Concentration mg/mL	Erythema intensity		Erythema size		Wheal size, maximum diameter	
		FLUX, PU	*P**-value	Area, cm	*P**-value	Wheal, mm	*P**-value
Morphine	0.5	503.95 ± 19.16	0.08	7.71 ± 2.36	0.93	11.5 ± 0.71	1
Morphine + Naloxone	0.5 + 0.2	458.49 ± 5.85		7.51 ± 1.7		11.5 ± 0.71	
Codeine	0.5	442.02 ± 14.95	0.48	10.74 ± 2.9	0.55	14.5 ± 2.38	0.9
Codeine + Naloxone	0.5 + 0.2	433.93 ± 15.19		12.22 ± 3.66		14.75 ± 2.75	
Meperidine	1	407.56 ± 42.02	0.89	6.58 ± 1.39	0.41	11.75 ± 1.26	1
Meperidine + Naloxone	1 + 0.2	411.41 ± 34.13		5.80 ± 1.05	11.75 ± 1.26		

One-way analysis of variance, $n = 6$, mean ± SD.
* $P < 0.05$ was considered as significant.

concentrations (both around 500 PU area under the curve), all others induced only a slight increase.

The joint injection of morphine (0.5 mg/mL), codeine (0.5 mg/mL), and meperidine (1 mg/mL) with naloxone (0.2 mg/mL) did not have any effect on the size and intensity of the developing axon-reflex flare, itch sensation, or on wheal formation mirroring protein extravasation (Table 1).

Discussion

Many tests have been developed, either *in vitro* or *in vivo*, to assess the ability of drugs to liberate histamine from mast cells. The harvesting of mast cells from rat peritoneal fluid and the incubation of these cells with the drug under surveillance has been extensively applied (14). Other *in vitro* approaches using human foreskin preparations have used release techniques to liberate histamine from skin mast cells (4). Some *in vivo* studies simply used the technique of intradermal injections of the respective drug, measuring the wheal and flare sizes bymeans of tracing their outlines (5). In our *in vivo* approach,we were able to simultaneously quantify local mediator release and their biological effects, thus providing more specific information and reducing the number of subjects required for each drug.

Under the conditions used in our experiments, the intradermal stimulation with the high-affinity μ receptor agonist morphine, with the low-affinity μ receptor agonist codeine, and with meperidine, which primarily shows κ-agonistic action, led to a dose-dependent increase of plasma extravasation, to the liberation of histamine and tryptase from cutaneous mast cells, to the subsequent induction of local vasodilation, to the formation of an axon-reflex erythema, and to distinct itch sensations. Systemic effects were not seen during the skin perfusion with even the largest concentrations of the most potent opioids. Considering the recovery experiments, in which recovery values between 25% and 35% were determined, approximately two-thirds of the concentration reached the skin of the subject, resulting in large local concentrations but only with a minor risk of systemic effects during the stimulation period.

Itch sensations experienced by the subjects ranged slightly more than the "scratch threshold" of 3 on the NRS of 0 to 10. This correlated well with the mast cell activation and the subsequent release of histamine and tryptase from the cytosolic granules (15). No other opioid induced any itch sensations. This correlated well with mast cell mediator levels ranging within control levels.

Similar to the observations of our previous study, in which the mast cell degranulatory effect of muscle relaxants was investigated, morphine, a high-affinity μ receptor agonist, codeine, the low-affinity μ receptor agonist, and meperidine all showed a very similar pattern of mast cell degranulation with a subsequent activation of C-nociceptive itch or pain fibers (16), vasodilation, and protein leakage from the vessels causing plasma extravasation (17). Again, none of the highly potent μ receptor agonists, the partial agonist buprenorphine, and the antagonist naloxone induced any of the effects related to neurogenic inflammation. Only the largest concentration of, for example, remifentanil (2.65 mM) provoked a slight increase of the local vasodilatory effect on the vessels located within the direct vicinity of the intradermal microdialysis membrane. Because no other effects, such as plasma extravasation, were seen, this was probably because of a direct action on the vessel walls, without increasing the permeability of the endothelial cell layer.

When intracutaneously administered, uncommonly large concentrations of, for example, fentanyl resulted in the formation of an axon-reflex erythema (5). In this study, the largest fentanyl concentration (0.3 mM) was double the normal clinically used concentration of 0.15 mM. All other opioids were administered in the widely used clinical concentrations, as described in Methods. Not even after the administration of the largest concentration of fentanyl did we see the induction of an axon-reflex flare or the formation of vessel leakage with subsequent plasma extravasation. In our study, the drug

was continuously administered after an equilibration period in which the skin was allowed to recover. The flow rate of the stimulation period was slow, excluding effects of rapid injection into the tissue.

Naloxone did not induce mediator release from skin mast cells or provoke neurogenic inflammation. In case of an involvement of μ receptors on either the skin mast cells or directly upon the vasculature, it would be expected that naloxone would inhibit the induction of mediator release from mast cells and the subsequent formation of an axon reflex erythema and plasma extravasation. Because mediator release and plasma extravasation did not occur during skin perfusion with highly potent μ receptor agonists, only the opioids morphine, codeine, and meperidine were co-administered with naloxone. Contrary to earlier publications postulating the involvement of μ receptors on the histamine release from mast cells leading to an axon-reflex flare (5), naloxone did not attenuate erythema, protein extravasation, or itch induced by the co-administered morphine, codeine, or meperidine.

Mast cells can be activated by immunological and by nonimmunological pathways. Both pathways lead to the liberation of preformed mediators such as histamine and mast cell tryptase with only minor differences in the release of prostaglandins or leukotrienes (18). In contrast to the immunoglobulin (Ig)E-mediated immunological activation, a concentration-dependent direct activation of pertussis-toxin-sensitive heterotrimeric G-proteins (g_{i2}, g_{i3}) by cationic drugs amidated at the C-terminus is the most likely mechanism of this nonimmunological pathway (19,20).

In conclusion, large concentrations of morphine and other opioids with lower affinity to the μ receptor induce the liberation of the mast cell mediators histamine and tryptase. In contrast, the potent μ agonists did not activate cutaneous mast cells *in vivo* or induce neurogenic inflammation and the formation of an axon-reflex flare.

These effects seen together indicate that the activation of skin mast cells by morphine, codeine, and meperidine does not depend on μ receptors but, more likely, on the direct activation of G-proteins of mast cells. Thus, rather than using μ-opioid antagonists, the inhibition of stimulated G-protein activity, for example, by cromolyn (20), seems to be the more promising approach. This could be tested by intradermally co-injecting or co-perfusing the skin with, for example, codeine and cromolyn.

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