Naloxone antagonizes the local antihyperalgesic effect of fentanyl in burnt skin of healthy humans

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RUNNING HEAD: Peripheral opioid antihyperalgesia

Abstract

The aim of this study was to investigate local opioid effects in the inflamed skin of healthy human volunteers. To induce inflammation, the circular tip of a 10-mm diameter probe was heated to 48ºC and applied for 120 s to a site on each forearm of 24 healthy participants. Thirty minutes later, 0.2 mL normal saline was injected subcutaneously into one inflamed site, and the opioid antagonist naloxone hydrochloride (80 μg in 0.2 mL) was injected subcutaneously into the other inflamed site. Participants completed tests of pain sensitivity (heat pain thresholds, heat pain ratings and mechanical pain ratings) before and after the injections. Fentanyl citrate (10 μg in 0.2 mL) was then injected into the pre-treated sites, and pain sensitivity was measured again. The thermal injuries produced thermal and mechanical hyperalgesia which did not differ between the saline and naloxone sites. After the fentanyl injections, decreases in thermal and mechanical hyperalgesia were greater at the saline site than the naloxone site. These findings demonstrate that pre-treatment with naloxone blocks local opioid effects produced by the subcutaneous injection of a low dose of fentanyl in the inflamed skin of healthy humans. Thus, peripheral opioid receptors could be a therapeutic target for painful cutaneous disorders.

Perspective: This article demonstrates that activation of opioid receptors in the skin inhibits sensitivity to painful mechanical and thermal stimuli. Thus, local application of low-dose opioid medications could relieve painful skin disorders.

Introduction

Opioids are usually administered systemically to control pain. However, opioid receptors are known to be present on cutaneous sensory nerves in rats[19] and humans.[18] The peripheral administration of opioids has been found to produce analgesia without systemic side effects in animals[15] and humans in response to clinical[4,12] and experimentally induced pain.[7,14] Peripherally administered opioids produce negligible analgesia in uninflamed tissue but potent analgesia in inflamed tissue.[19]

Experimental procedures that do not injure tissue, such as intradermal injection or topical application of low doses of capsaicin,[7,8] sensitize nociceptors and have been useful models of neurogenic inflammation. However, procedures that damage tissue, such as burning the skin,[16] freezing the skin or pinching the interdigital web,[5,6] more closely represent inflammatory pain. Experimentally-induced cutaneous burns produce reliable effects on pain sensitivity, the magnitude of which is repeatable within participants.[16] Controlled burns induce thermal hyperalgesia and can also evoke mechanical hyperalgesia, but this appears to require a more severe burn than hyperalgesia to heat.[2]

Opioids administered following experimentally-induced burn injury in humans consistently reduce burn-induced hyperalgesia.[9,14] Such antihyperalgesia appears to be more pronounced for thermal than mechanical stimuli. Koppert et al.[9] examined the effect of peripheral opioids in skin burnt by ultraviolet irradiation. One day after the induction of inflammation, the blood supply to the arm was limited by the inflation of a pressure cuff to ensure that any opioid effect was peripheral. Morphine hydrochloride (4 mg in 40 mL) was then administered intravenously to the burnt site. Morphine reduced thermal

pain but not mechanical pain sensitivity, an effect that apparently was mediated by a local mechanism because the concentration of morphine and morphine metabolites in the systemic circulation was insufficient to have induced analgesia centrally. Moiniche, Dahl, and Kehlet[14] investigated the antinociceptive effect of 2 mg morphine injected subcutaneously at a site of thermal injury. Approximately 30 min later, thermal and mechanical pain thresholds were greater at the morphine site than at a burnt site injected with saline. In addition, most participants reported that the morphine-treated site was less sensitive than the saline-treated site.

The antihyperalgesia described in these studies was assumed to be the product of peripheral opioid receptor activation. However, this must remain speculative in the absence of studies demonstrating blockade of antihyperalgesia by an opioid receptor antagonist. Thus, the aim of the present study was to determine whether the opioid receptor antagonist naloxone would block the local antihyperalgesic effect of the mu-opioid receptor agonist fentanyl in the inflamed skin of healthy volunteers. It was hypothesized that the subcutaneous injection of fentanyl into burnt skin would reduce hyperalgesia to thermal and mechanical stimuli, and that pre-treatment with naloxone would reduce the potency of this antihyperalgesia.

Methods

Participants

Participants were 24 healthy volunteers (nine men and 15 women) whose ages ranged from 17 to 39 yr (median = 25.5 yr). Participants were advised not to drive a car for 6 hours following the procedures, and were reimbursed a small sum of money for their time and travel costs. Exclusion criteria were pregnancy and narcotic addiction,

which were assessed by self report. Participants gave written informed consent to complete the procedures, which were approved by the ethics committee at the University of Western Australia.

Procedure

Room temperature ranged between 22.6 and 26.1ºC (M = 24.2 ºC). Two test sites, 8 and 15 cm proximal to the crease of the wrist on the dorsal aspect of each arm, were marked with ink. A burn injury was produced at the distal site on each arm in half of the participants (selected at random), and at the proximal site in the remainder. A purpose-built thermocouple-controlled cautery unit was used to produce burn injuries. The circular tip of a 10-mm diameter probe was heated to 48ºC and applied for 120 s with a force of approximately 1 N to one site on each arm.[2] Participants were given a 30-min break to allow pain sensitivity to stabilize.

Heat pain thresholds (HPTs) were measured using a heat lamp which directed radiant heat from a halogen globe through a lens and circular aperture (1.1 cm in diameter) onto the skin. Skin temperature was measured by a thermistor, which was positioned under an aluminum shield in the center of the aperture. The arm of the lamp could be adjusted to allow the thermistor to touch the skin lightly without transferring the weight of the lamp. Skin temperature was held at a baseline of 35.6ºC for 10 s and then increased linearly by 0.5ºC/s. In the hand contralateral to the stimulated arm, participants held a button which they were instructed to press when they first felt pain. The lamp switched off when the button was pressed or the temperature reached 47ºC. The HPT estimate was the temperature at which the lamp switched off. Two HPT estimates were

conducted at each site, with a third administered if the first two differed by more than 2ºC (14% of trials). For each site, the mean of the 2-3 HPT estimates was recorded.

Heat pain ratings (HPRs) were made in response to the application of the heat lamp at 45ºC for 5 s. Participants rated pain severity using a computerized 10-cm visual analogue scale (VAS), marked only with the endpoints "no pain" and "worst pain ever". Ratings were measured to the closest mm. Mechanical pain ratings (MPRs) were made in response to the application of a standard von Frey filament with a bending threshold of 121 mN. To increase sharpness, the angle at the tip of the filament was decreased to approximately 45º, reducing the contact surface area to about 0.5 mm^2. Participants rated pain on the computerized VAS in response to a single application of the von Frey filament for approximately 1 s on each trial. Two HPR and MPR trials were conducted on each measurement occasion, and the mean rating at each site was recorded.

Participants were initially given four practice trials of HPTs, HPRs, and MPRs at non-test sites. Participants wore an eye mask during stimulus delivery. Baseline pain-sensitivity measurement began 30 min after the first burn injury at the burnt sites and an untreated site on each arm.

Participants then washed their forearms with soap and warm water and dried them with a paper towel. Burn sites were further cleaned with an alcohol wipe, and 0.2 mL normal saline was injected subcutaneously into one burn site and naloxone hydrochloride (David Bull Laboratories, 80 μg in 0.2 mL) into the other. Injections were administered double-blind and randomized to prevent order effects. Pain sensitivity measures commenced five minutes after the injections to determine whether naloxone alone had any effect on local pain sensitivity. Fentanyl citrate (Sigma, 10 μg in 0.2 mL normal

saline) was then injected subcutaneously into each burn site. Injections were administered subcutaneously to avoid the bleb produced by intradermal injections which might interfere with pain sensitivity measures. Subcutaneous injections have been used previously to investigate local opioid effects.[14] The 10 μg dose of fentanyl in 0.2 mL saline was based on extensive pilot studies that established that a smaller dose or a greater injection volume was ineffective in countering hyperalgesia.[17] The 80 μg dose of naloxone was also based on pilot studies.[17] In humans, systemic administration of high doses of naloxone (7.5 and 10 mg) induces hyperalgesia whereas low doses (0.4 and 2 mg) induce analgesia;[10] however, very low doses (40 μg) have no systemic effect.[20] Local injection of naloxone at doses as low as 5 μg in the inflamed rat paw blocks the local antihyperalgesic effect of opioid agonists.[1] Pain sensitivity was measured again at the experimental sites starting 5 min after the fentanyl injections. Total testing duration was approximately 1.75 hr.

All HPT, HPR, and MPR data are displayed as means ± standard error of the mean. Separate 2 (drug: saline pre-treatment, naloxone pre-treatment) x 2 (phase: before and after fentanyl) repeated measures analyses of variance were conducted to investigate changes in HPT, HPR, and MPR after the fentanyl injections. Significant drug x phase interactions were investigated further with paired t-tests, to determine whether the antihyperalgesic effect of fentanyl was greater at the saline-pretreated site than the naloxone-pretreated site. A Bonferroni correction was not necessary for these t-tests because only two sites were compared.

Results

Effect of burn injury on pain sensitivity

As shown in Table 1, the burn injuries produced moderate hyperalgesia to heat and weak hyperalgesia to mechanical stimulation. In particular, the mean HPT at the burnt sites was lower than the mean HPT at the untreated sites, and the mean HPR and MPR at the burnt sites were greater than the mean HPR and MPR at the untreated sites. The effect of the burn injury on thermal and mechanical pain sensitivity was consistent across both burnt sites.

Effect of fentanyl on pain sensitivity at sites pre-treated with saline or naloxone

The HPT did not differ significantly between the saline and naloxone sites before the fentanyl injections, but the HPT increased after the fentanyl injections at the site pre-treated with saline (Figure 1). The main effect of phase was significant, F (1,23) = 5.93, p < .05, showing that, overall, HPT increased from pre- to post-fentanyl injections. This main effect was subsumed within a significant drug by phase interaction, F (1,23) = 6.74, p < .05, indicating a differential effect of the fentanyl injection at the saline and naloxone sites on HPTs. The HPT increased significantly from pre- to post-fentanyl injection at the saline site (M [pre-fentanyl] = 42.1ºC, M [post-fentanyl] = 42.9ºC), t (23) = 4.64, p < .001, but not at the site pre-treated with naloxone.

The HPR did not differ significantly between the saline and naloxone sites before or after the fentanyl injections (Figure 2). The main effect of phase approached significance, F (1,23) = 4.11, p = .05, suggesting an overall reduction in HPRs from pre- to post-fentanyl injection. The interaction between drug and phase was not significant, F

(1,23) = 2.30, p = .14, providing no evidence that the reduction in HPRs following the fentanyl injections differed between the saline and naloxone sites.

The MPR did not differ significantly between the saline and naloxone sites before the fentanyl injections (Figure 3). Following the fentanyl injections, the mean MPR decreased at the saline site (*M* [pre-fentanyl] = 2.9 cm, *M* [post-fentanyl] = 2.5 cm) relative to the naloxone site (*M* [pre-fentanyl] = 2.7 cm, *M* [post-fentanyl] = 2.8 cm), interaction between drug and phase, F (1,23) = 5.37, $p < .03$. The MPR reduction from pre- to post-fentanyl approached significance at the saline site, t (23) = 1.92, p = .07, whereas the increase was not significant at the naloxone site. These results indicate that fentanyl reduced MPRs when injected at a burnt site pre-treated with saline relative to a burnt site pre-treated with naloxone.

Discussion

The main finding of the present study was that local pre-treatment with the opioid antagonist naloxone blocked the antihyperalgesic effects of the mu-opioid agonist fentanyl on HPT and MPR. However, contralateral pre-treatment with naloxone did not block the antihyperalgesic effects of fentanyl. Thus, the antihyperalgesia induced by fentanyl apparently was mediated locally by mu-opioid receptors on peripheral nerves.[18,19] These findings are consistent with research conducted on non-human species that identified peripheral opioid effects in inflamed and burnt skin.[15,19] Our procedures did not permit us to ascertain whether the injections of fentanyl also acted centrally to inhibit pain. However, this seems unlikely because hyperalgesia did not decrease significantly at the naloxone-pretreated site after the fentanyl injections.

We chose to employ a mild burn to avoid tissue damage and blisters that might complicate the interpretation of findings. In contrast, Moiniche et al.[14] heated the skin to 49^{o}C for 5 minutes, resulting in blisters and second-degree burns. Koppert et al.[9] irradiated the skin with ultraviolet B light and studied the resultant hyperalgesia 24 hours later. To ensure that intravenously administered morphine did not exert any systemic effect, the blood supply to the hyperalgesic limb was blocked for 20 min with a cuff at 250 mm Hg while pain sensitivity was determined. Cuff pressure may have had effects on pain sensitivity that were independent from those of ultraviolet irradiation (e.g., potentiation of hyperalgesia due to accumulation of lactic acid, or attenuation of hyperalgesia due to pressure block of peripheral nerves). Furthermore, the use of morphine in both of these studies complicates the methodology as morphine induces mast cell degranulation and consequent pro-inflammatory effects.[3,11] Despite these methodological shortcomings, morphine clearly inhibited thermal hyperalgesia in burnt skin.

The present results extend these findings by demonstrating that fentanyl-induced antihyperalgesia was mediated peripherally by opioid receptors. Pre-treatment of burnt skin with a subcutaneous injection of naloxone reduced the antihyperalgesic potency of fentanyl for HPTs and MPRs. Naloxone weakened the antihyperalgesic effects of fentanyl locally but not in the contralateral arm, providing strong evidence that the antihyperalgesic effects of fentanyl were mediated by opioid receptors on peripheral nerves. However, hyperalgesia did not differ between sites immediately following the naloxone and saline injections, implying that there was insufficient release of endogenous opioids in the skin to influence pain sensitivity.

The HPT increased almost 1°C after the 10 µg fentanyl injection at the saline-pretreated site. Moiniche et al.[14] also reported that the HPT increased by approximately 1°C after subcutaneous injection of 2 mg morphine in burnt skin, whereas Koppert et al.[9] found that the HPT increased by around 1°C in moderately inflamed skin and 2°C in more severely inflamed skin ten minutes after intravenous regional injection of 4 mg morphine. In contrast, naloxone did not influence HPRs after the fentanyl was injected, suggesting that HPRs are less sensitive to local opioid effects than HPTs.

One limitation of the present study is that the sample was too small to investigate gender differences in the response to fentanyl. This might be important, because females appear to be more sensitive than males to drugs that act at opioid receptors.[21] Another limitation is that the 2-min application of a 48ºC heat probe produced weak mechanical hyperalgesia and only moderate thermal hyperalgesia. A more severe thermal injury that better resembles clinical pain conditions might allow a clearer demonstration of peripheral opioid blockade, particularly for mechanical hyperalgesia.

Although pharmacological blockade of local opioid-mediated antihyperalgesia has been demonstrated in rodents,[15] to our knowledge this is the first demonstration that opioid-receptor blockade antagonizes the local antihyperalgesic effects of fentanyl in healthy humans with experimentally-induced pain. Thus, the induction of burn injuries produced by the 2-min application of a 48ºC probe can be used as a model of inflammatory pain in healthy humans that allows locally administered opioids to activate peripheral opioid receptors. Compared to studying peripheral nociceptive mechanisms in individuals with clinical pain, the study of peripheral opioid analgesia in pain-free humans allows greater control over the injury, is cheaper to conduct, and provides greater

experimental sensitivity because participants can serve as their own control. Such a model would allow further investigation of the conditions under which the peripheral opioid system is active in healthy humans. Furthermore, this study supports the efficacy of peripherally applied opioids for the relief of localized inflammatory pain.[13] Further clinical studies are needed to investigate the therapeutic effect of subcutaneously administered fentanyl for individuals with painful cutaneous disorders.

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Table 1

Effect of the thermal injury on thermal and mechanical hyperalgesia

	Mean ± S.E.		t-test
	Burnt Sites	Unburnt sites	(23 d.f.)
Heat pain threshold (^{o}C)	41.42 ± .41	43.55 ± .42	7.55 ***
Heat pain rating (cm)	3.38 ± .52	1.42 ± .30	6.00 ***
Mechanical pain rating (cm)	2.05 ± .37	1.77 ± .34	2.31 *

Difference between the burnt and unburnt sites statistically significant: *** $p<0.001$; * $p<0.05$

Figure legends

Figure 1. Mean heat pain threshold (in ºC) at a burnt site treated with saline and then fentanyl, and a burnt site on the contralateral arm treated with naloxone and then fentanyl. The heat pain threshold increased significantly after the fentanyl injection at the site pre-treated with saline (* $p<0.001$) but not at the site pre-treated with naloxone. In Figures 1-3, error bars represent ± 1 standard error of the mean (n = 24).

Figure 2. Mean heat pain rating (on a 10-cm VAS) to a 45^{o}C stimulus for 5 s at a burnt site treated with saline and then fentanyl, and a burnt site on the contralateral arm treated with naloxone and then fentanyl.

Figure 3. Mean mechanical pain rating (on a 10-cm VAS) to a 121 mN bristle at a burnt site treated with saline and then fentanyl, and a burnt site on the contralateral arm treated with naloxone and then fentanyl. After the fentanyl injection, the mechanical pain rating decreased at the saline-pretreated site relative to the naloxone-pretreated site ($p<0.05$).

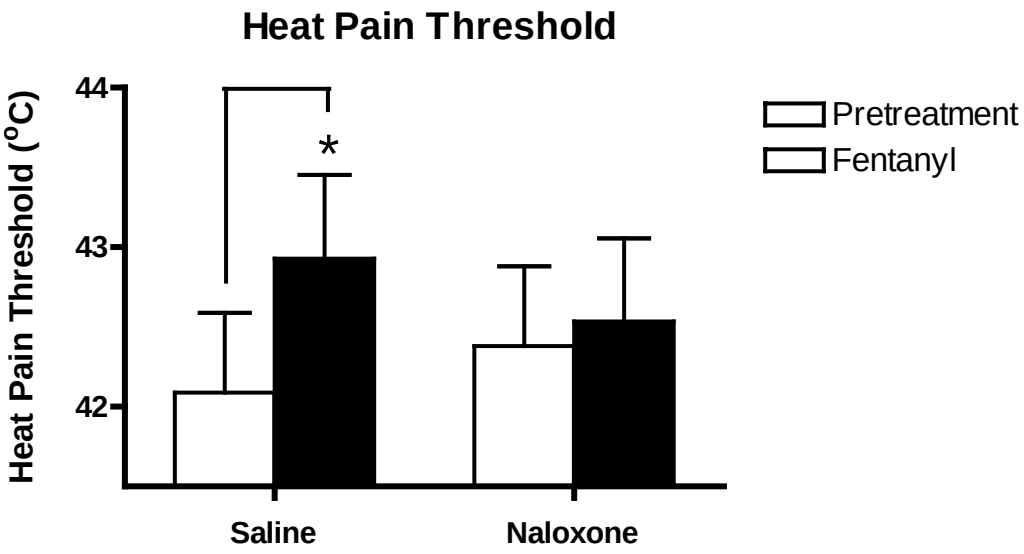
Heat Pain Threshold
Heat Pain Threshold (°C)
44
43
42
*
Saline
Naloxone
Pretreatment
Fentanyl

Figure 1.

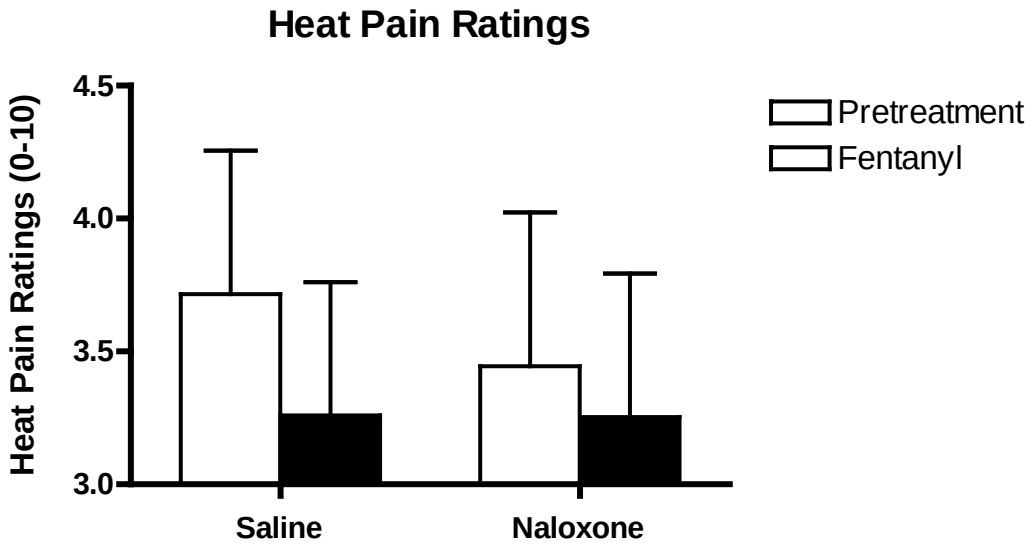
Heat Pain Ratings
Heat Pain Ratings (0-10)
4.5
4.0
3.5
3.0
Saline
Naloxone
Pretreatment
Fentanyl

Figure 2.

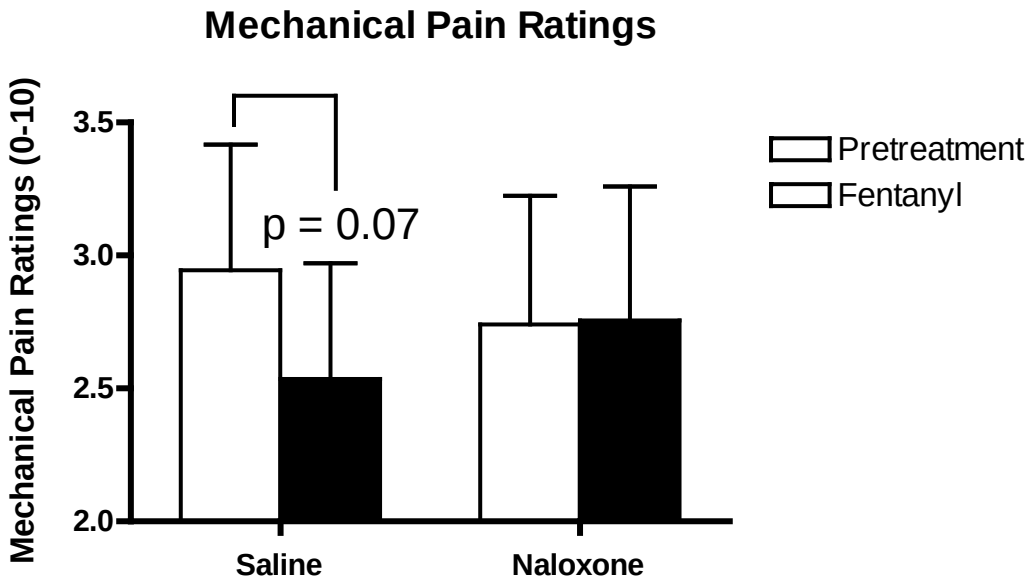
Mechanical Pain Ratings
Mechanical Pain Ratings (0-10)
3.5
3.0
2.5
2.0
p = 0.07
Saline
Naloxone
Pretreatment
Fentanyl

Figure 3.