Opioid treatment of experimental pain activates nuclear factor-κB

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Abstract

Objective—To determine the independent and combined effects of pain and opioids on the activation of an early marker of inflammation, nuclear factor-κB (NF-κB).

Design—NF-κB activation was compared within-subjects following four randomly ordered experimental sessions of opioid-only (intravenous fentanyl 1 μg/kg), pain-only (cold-pressor), opioid + pain, and a resting condition.

Setting—University General Clinical Research Center.

Participants—Twenty-one (11 female) healthy controls.

Interventions—Following exposure to treatment (fentanyl administration and/or cold-pressor pain), blood samples for NF-kB analysis were obtained.

This paper was presented orally at the American Pain Society 33rd Annual Scientific Meeting at the Tampa Convention Center, Tampa, FL on May 1, 2014.
Main outcome measures—Intracellular levels of activated NF-κB, in unstimulated and stimulated peripheral blood mononuclear cells at 15 and 30 minutes.

Results—Neither pain nor opioid administration alone affected NF-κB levels in cell populations; however, the combination of treatments induced significant increases of NF-κB in stimulated peripheral blood mononuclear cell, lymphocytes, and monocytes.

Conclusions—The combination of acute pain with opioids, as occurs in clinical situations, activates a key transcription factor involved in proinflammatory responses.

Keywords
pain; opioids; NF-κB; cold-pressor test; fentanyl

INTRODUCTION

The process of inflammation provides the foundation for healing. A primary function of the innate immune system is inflammation, a nonspecific and immediate response to cellular injury or invasion. It is initiated by mast cells located next to vessels and serves to carry specific proteins, fluid, and cells to injured tissues. Via activation of phagocytic cells, potential infective causative agents and damaged tissue are destroyed and eliminated, removing cellular debris from site of damage. Additionally, the inflammatory process provides signals that initiate adaptive immunity responses and activate soluble protein systems (including clotting).

Pain is a companion of inflammation; not only are nociceptive mediators (ie, bradykinin and prostaglandins) produced during the inflammatory process, but recent evidence suggests that via interactions with the sympathetic nervous system, the experience of acute pain itself can activate proinflammatory markers of the innate immune system. Various experimental pain induction modalities (heat, mechanical, electrical stimulation, and cold-pressor) have been shown to increase catecholamines, and certain intra-cellular (nuclear factor [NF]-κB) and cytokine (IL-6) markers of proinflammatory activity, suggesting that the stress response associated with pain can affect inflammatory processes.

Fortunately, clinicians have available a variety of safe and effective analgesics with which to treat pain, the opioids being among the most reliable and powerful. With respect to the immune system, opioids have repeatedly been demonstrated to have broad immunosuppressant effects, attributed to decreases in macrophage activity, and interfering with production and release of cytokines necessary to mount an effective inflammatory response. Diminished NF-κB activation in stimulated immune cells in response to opioids (morphine and fentanyl) has been reported in several in vitro examinations. Although the clinical relevance of this opioid-induced immuno-suppression is disputed, the association between opioid use and postoperative infection continues to be a concern.

Thus, pain and opioids appear to exert independent and contradictory effects on immune system activity. Translating to the clinical setting, patients not uncommonly present with both acute pain combined with opioid analgesia. No study to date has examined the...
combined effects of opioid administration and pain on inflammatory signaling in humans. The purpose of this study was to characterize the responses of an early, proinflammatory, intracellular transcription factor, NF-kB, to acute pain and opioid analgesia, separately and together, in healthy control subjects. Examined were the short-term (15 and 30 minutes) main and interaction effects of opioid administration (fentanyl challenge) and experimental pain induction (cold-pressor) on the expression of the NF-κB in peripheral blood mononuclear cells (PBMC), and their two major subpopulations, lymphocytes and monocytes.

**METHODS**

The study was preliminary and observational, using a nonblinded, randomized design. Evaluated were intracellular levels of NF-κB in response to an acute pain stimulus and/or an opioid challenge as compared to a resting condition. Specifically, on separate study days, an opioid analgesic challenge (fentanyl intravenous [IV] 1 μg/kg) and/or a standard experimental acute pain stimulus (cold-pressor test [CPT]) were administered to all subjects. Each subject underwent four randomly ordered study sessions: opioid-only (O); pain-only (P); opioid + pain (OP), or a resting control session (C) during which neither pain nor opioid was administered. Study sessions were scheduled at least 48 hours apart to rule out carryover effects of pain and/or opioid administration.

**Sample**

Healthy controls were recruited from the university community using institutional review board (IRB)-approved advertising. To minimize age-related differences in pain perception and immune response, only individuals aged between 21 and 40 years were included. Eligible subjects were in good general health, fluent in English, and expressed willingness to participate in the study. Exclusion criteria included the regular use of any medication with effects on immune system activity or pain perception (including opioids); known hypersensitivity to opioids; pregnancy; presence of acute or chronic pain syndrome; peripheral neuropathy, neuropsychiatric illness (ie, mood disorder and schizophrenia) known to affect pain perception; a current or past history of high blood pressure (BP) or heart disease; or presence of chronic immune compromise or acute infection within the last 4 weeks.

**Procedures**

**Screening**—Prior to signing the IRB-approved study consent, potential participants underwent a general medical and psychiatric evaluation, including electrocardiogram, blood draw for a chemistry panel, complete blood and immune cells counts, urine screen for drugs of abuse, report of medical or psychiatric illness requiring ongoing treatment, and report of concomitant (including over-the-counter) medications. Each was carefully interviewed to establish cardiovascular health, psychiatric and cognitive status, and previous opioid exposure. Females were tested for pregnancy. Screening took place in a private clinical office by a research nurse practitioner under the oversight of the study physician. Twenty-five participants (13 female) were deemed eligible for the study; all had been exposed to opioids at some point prior to study admission, but none reported regular or recreational use
of opioids. Of these, four subjects withdrew early, either completing no sessions (n = 2) or completing the first session only (n = 2), thus all data analyses reported here include the 21 subjects who completed all four study sessions. Once consented, subjects were randomly assigned to the order of study sessions. Prior to beginning study procedures, all participants were given a practice session of the CPT pain induction procedures, allowing familiarization with the method and processes involved. To encourage study participation and protocol compliance, participants were compensated for the screening session and each study session completed.

**Study sessions**—All sessions took place in a medically monitored private room at the UCLA Clinical Translational Research Center, where therapeutic oversight and emergency equipment were available at all times. For all study procedures, subjects reclined in a standard hospital bed; vital signs and responses were continuously monitored. Sessions began at approximately 8:00 AM each morning, and subjects were instructed to ingest no caffeine for 1 hour prior to each study session.

To ensure subjects were in a stable condition, a brief health screening (including urine human chorionic gonadotropin for female subjects), and Beck Depression Inventory (BDI) and Hamilton Anxiety Scale (HAS) were completed prior to study procedures. IV access in the dominant arm was established for blood sampling and opioid administration, with the nondominant limb used for the CPT. Continuous monitoring equipment was applied 30 minutes prior to collecting the baseline blood samples.

Baseline measures were collected approximately 30 minutes prior to CPT (Table 1). To allow for stable blood levels, opioid administration took place 15 minutes prior to the CPT (T = 0) during the O and OP sessions. For the P and OP session, the CPT commenced at T = 0. Before leaving the study sessions, subjects were evaluated to ensure that no residual effects remained. In the resting condition (C), an IV catheter was placed, but subjects received neither CPT nor opioid, and reclined comfortably, reading or watching TV, for the entire session.

**Opioid administration (fentanyl)**—The opioid administered in conditions O and OP was a single IV dose of fentanyl (1 μg/kg). Fentanyl (Sublimaze®) is a potent ultra-short-acting synthetic opioid with estimated IV potency 81 times that of morphine. It was selected for the opioid challenge due to its immediate onset and short duration of action (1 hour); the dose chosen approximates the lower end of the recommended dose (1–2 μg/kg IV bolus) for the treatment of acute pain. Hospital formulary fentanyl was diluted to a constant volume of 2.0 mL, and administered over slow (2–3 minutes) IV push by licensed nursing personnel. Pulse oximetry was used to monitor for respiratory depressant effects, with naloxone available if necessary.

As evidence of subjective opioid effects, responses to a modified Opioid adjective checklist were collected immediately following the CPT in the OP session and at 30 minutes following peak opioid effect (T0) in the O condition. The self-administered checklist consists of 10 items rated on a scale from 0 (not at all) to 4 (extremely): “flushing,” “skin itchy,” “sweating,” “turning of stomach,” “dry mouth,” “drive (motivated),”
“carefree,” “good mood,” “nodding,” and “vomiting.” Participants were kept an additional 4 hours after the last study measure to ensure the opioid effects had subsided. Prior to discharge, they were instructed not to operate a motor vehicle 24 hours following the fentanyl administration, and before beginning study procedures, nursing staff confirmed participants were not driving home after the session. There were no adverse events related to opioid administration.

**Acute pain induction (cold-pressor test)—**The experimental stimulus for acute pain in conditions P and OP was provided by a standardized CPT adapted for the pharmaceutical industry by Eckhardt et al. The intense cold reliably produces an acute and tonic noxious cold pain stimulus, activating peripheral nociceptors and central pain systems, and is accompanied by a well-described sympathetic nervous system and immune system response.

Subjects were seated comfortably in front of two plastic containers, one filled with warm (37.8°C) and one with cold (1.0 ± 0.5°C) water. A water pump in the cold container prevented laminar warming around the immersed limb. A BP cuff was applied to the nondominant arm, and a blindfold was secured over the eyes to reduce distraction. Other than instructions, subjects were not spoken to during testing. The forearm was immersed in the warm water with fingers spread wide apart with instructions not to touch the container, and timing began.

At 105 seconds, the BP cuff was inflated to 20 mm Hg below the diastolic BP to induce mild ischemia in the limb prior to determining the reaction to cold. At exactly 2 minutes, subjects were assisted in removing the forearm from the warm water container and fully immersing it with fingers spread wide into the cold-water container, not touching sides or bottom. Subjects were instructed to say “pain” when the cold sensation became painful (threshold), and to keep the limb immersed until the pain was intolerable or immersion time reached 300 seconds, at which point the arm was removed (tolerance), and a warm towel was provided. Threshold and tolerance were measured in seconds of immersion. Subjective ratings of pain severity and stress associated with the pain were collected immediately following each CPT trial using visual analogue scales (VAS), ranging from 0 (very mild, not stressful) to 10 (very severe, very stressful), thus, the precise timing of these measures varied with each individual and session.

**Assessment of NF-κB levels—**Heparinized blood samples were collected at baseline, and 15 and 30 minutes after initiation of CPT and/or peak fentanyl effect, to purify PBMC to capture early evidence of activation of NF-κB. PBMC were purified by Ficoll density centrifugation, and resuspended in phosphate-buffered saline (PBS) at 1 × 10^6 cells/mL. Aliquots of 1 × 10^6 cells were either left unstimulated or stimulated with 10 ng of recombinant human TNFα (R&D Systems), and incubated for 15 minutes at 37°C. Each PBMC aliquot was then fixed in a final concentration of 2 percent paraformaldehyde and frozen at −80°C. After the completion of all four experimental sessions by a subject, PBMC were thawed, washed (PBS with 0.5 percent bovine serum albumin, 0.1 percent sodium azide), and treated with 90 percent methanol for 30 minutes on ice to permeabilize the nuclear membrane. PBMC were washed again, then stained (10 μL antibody/0.5 × 10^6 cells,
60 minutes at room temperature in the dark) with phycoerythrin-labeled monoclonal antibody specific for the phosphorylated (activated) serine 529 (pS529) in the transactivation domain of human NF-κB p65 (BD Biosciences). Stained PBMC were analyzed by single color flow cytometry using CellQuest software (BD), gating on total PBMC, lymphocytes only, or monocytes only, based on forward versus side scatter. The amount of activated NF-κB signaling was expressed as the mean fluorescent intensity (MFI) of the population of cells being analyzed. All PBMC samples from all sessions from a single individual were stained and analyzed by flow cytometry in the same batch, so the MFIs within an individual can be compared directly to one another. All batches of samples were analyzed using the same instrument and settings.

Data analysis

Basic descriptive statistics were used to describe participants with respect to fundamental characteristics such as age, ethnicity, education, and employment factors. Paired t-tests were used to investigate the difference in the means of cold-pressor pain responses when subjects received pain alone and with opioid. Repeated-measures analysis of variance (ANOVA) models (mixed models) were used to evaluate the effects of treatment (C, P, and O) on the outcome variables adjusting for baseline, gender, age, and time point as compared to the opioid and pain condition (OP). A p-value of <0.05 was considered as a significant difference in the means.

RESULTS

Table 2 indicates that the sample was fairly representative of the university community with respect to subject age, marital status, and years of education. Approximately half were female, and baseline psychological measures (HAS and BDI) were similar among subjects and stable across sessions, demonstrating low overall rates of psychological distress in the sample (data not shown).

Pain and opioids

Both pain and opioid + pain administration had robust effects on nociceptive and subjective measures in the expected direction. During the pain-only (P) session, significant increases in systolic BP (p = 0.008), mean BP (p = 0.047), and respiratory rate (p = 0.034) were present immediately following the CPT, consistent with sympathetic activation (Table 3). In contrast, no significant increases from baseline were seen following CPT when the subject was pretreated with fentanyl (OP session) (data not shown). As expected, CPT pain threshold (p = 0.025) and tolerance (p = 0.002) times were significantly shorter (indicating faster/more distress) in the P than the OP session (Table 4). The percentage of subjects who were able to tolerate the maximum cold-pressor tolerance time (300 seconds) during the P session (14 percent) more than doubled in the OP condition (33 percent).

Mean pain severity and stress VAS ratings post-CPT were low, but with significant variability; means were somewhat lower in the OP condition but not significantly different from the P condition (Table 4). In both P and OP conditions, neither severity nor stress VAS ratings correlated with CPT pain threshold (r = −0.03) or tolerance (r = −0.04). Opioid
adjective checklist responses did not differ between the O and OP conditions. Few symptoms were reported; those endorsed were consistent with expected psychoactive opioid effects (motivated, carefree, and good mood) (data not shown).

Activation of NF-κB

In unstimulated cells, intranuclear levels of activated NF-κB did not differ between single or combined treatment conditions in the PBMC (F(3, 216.6) = 0.25, p = 0.86), lymphocytes (F(3, 281.6) = 0.52, p = 0.67), or monocyte (F(3, 213.8) = 1.67, p = 0.18) population. Pairwise comparisons in unstimulated monocytes showed significantly higher levels of NF-κB activation in opioid-only (O) session as compared to pain-only (P) conditions (p = 0.03).

However, levels of activated NF-κB were significantly different between conditions in stimulated PBMC (F(3, 214.7) = 2.4, p = 0.06), monocytes (F(3, 214.2) = 3.0, p = 0.03), and lymphocytes (F(3, 214.9) = 2.4, p = 0.06). Pairwise comparisons demonstrated significantly greater change in levels of NF-κB in stimulated PBMCs in OP vs P (p = 0.034), and the OP vs O (p = 0.018) conditions (Figure 1a). Similarly, in stimulated monocytes, data showed significantly higher levels of activated NF-κB in OP vs P (p = 0.044) and OP vs O (p = 0.004) sessions (Figure 1b). In stimulated lymphocytes, pairwise comparisons also demonstrated significantly greater change in levels of NF-κB in OP vs P (p = 0.034) and OP vs O (p = 0.018) sessions, as well as OP vs the control condition (C) (p = 0.045) (Figure 1c). Mixed model analyses indicated that the results did not differ controlling for gender or age (data not shown). Thus, when pain was combined with opioids, mean change of activated NF-κB in stimulated cells was significantly greater than when either was administered alone.

DISCUSSION

Pain and opioid responses

Subject responses to cold-pressor pain and opioid administration were consistent with expectations and demonstrated clear treatment effects. The CPT activated a sympathetic nervous system response, consistent with the wide body of literature demonstrating that the technique results in release of catecholamines and cortisol. In addition, performance on the CPT indicated that subjects had no difficulty identifying pain threshold and tolerance, and immersion times were well-within normal range. However, subjective ratings of pain severity and stress were low throughout the study, indicating that the treatment effect of pain may not have been sufficiently stressful to mount an appreciable NF-κB response.

The analgesic effect of opioids was evident in producing decreased CPT pain severity and stress ratings, and significant improvements in pain threshold and tolerance. The presence and degree of subjective opioid effects were generally similar across the O and OP study sessions, and reflective of an opioid agonist response. Contrary to previous investigations, acute pain did not appear to interfere with the subjective effects of opioids.
NF-κB responses

Contrary to previous studies, neither experimental pain nor opioid alone had appreciable effects on NF-κB activation in simulated or unstimulated PMBCs. The single exception was NF-κB activation being greater in the O vs P sessions in unstimulated monocytes, but neither differed from control responses.

Specifically, increases in NF-κB in response to pain were not supported. It is likely that these negative findings are related to the relative lack of stress associated with the pain stimulus. The cold-pressor pain was not experienced as especially severe and did not correlate to pain threshold or tolerance. For most subjects, VAS pain severity scores did not reach levels that would typically be treated with opi-oids in the clinical situation. It appears that the notable changes in BP and respirations reflect a sympathetic response to the CPT, but not necessarily a pain, response. Hypothesizing that pain severity, and therefore associated psychological stress, is requisite to induce pain-related proinflammatory responses, it is not surprising that NF-κB activation was not affected in this study.

Similarly, no changes in NF-κB activation were observed with opioid administration alone, despite evidence suggesting that opioid agonists decrease activity of NF-κB in stimulated cells. While the fentanyl dose was sufficient to relieve the pain associated with the cold-pressor stimulus, and comparable to doses used clinically, it was not sufficient to induce changes at the level of transcription factor activation. Recent in vitro work suggests that fentanyl is distinct from other opioids, having a relative lack of effect on certain proinflammatory systems (TNF-α, IL-8). This suggests that a different challenge opioid may induce a greater NF-κB response.

It has also been argued that opioid effects on NF-κB activity are unrelated to opioid agonist activity. For example, in a murine model of induced acute peritonitis, the effect of morphine on the expression of NF-κB was mediated by activity at the toll-like receptors. Implicate L-type calcium channels in affecting the same. Others have shown that tramadol is a more potent inhibitor of cytokine release and NF-κB activation than are the pure opioid agonists, suggesting that norepinephrine or serotonin systems may underlie immunosuppressant responses. Further supporting a nonopioid mechanism, Bastami et al. showed that several opioid-induced anti-inflammatory effects were not reversed by naloxone, and, recent work done in laboratory of Mizota show that administration of naloxone itself can affect NF-κB expression. Thus, opioid agonist activity alone may not be sufficient to induce changes in NF-κB, as this relies on processes in nonopioid agonist systems.

Also not supported are reports of acute opioid administration increasing circulating levels of several proinflammatory cytokines, including NF-κB. For example, within hours of heroin or morphine administration, mice demonstrate increased serum levels of IL-6 and splenocyte production of IL-1β, IFN-γ, IL-12, and TNFα, effects antagonized by naltrxone. Proinflammatory consequences are suggested by parallel evidence for decreased expression of the anti-inflammatory cytokines, IL-10 and IL-4, following acute opioid exposure. Further, via toll-like receptors, opioids induce proinflammatory effects on spinal glial cells, which is thought to lead to increases in cytokines in the plasma. Neither
a proinflammatory nor an anti-inflammatory response to opioid exposure was observed in the current data.

When opioid and pain were paired in stimulated cells, robust changes were evident. Being an early indicator of proinflammatory activity, effect sizes on NF-κB activation were largest at 15 minutes post-treatment, with PBMC r = 0.87, monocytes r = 0.83, and lymphocytes r = 0.89. Medium effect sizes were evident at 30 minutes for all measures (PBMC r = 0.51; monocytes r = 0.40; and lymphocytes r = 0.52). These results stand in contrast to those observed in unstimulated cells, in which no pattern of significant change was noted in the opioid-pain condition. Hypothesizing that the stimulated condition better models tissue injury, the clinical implications of the finding are highlighted.

Because of the limitations of the study (see below), it is difficult to interpret the strong interaction effect vis-à-vis the lack of main effects. These data suggest that neither pain nor opioid alone affects NF-κB activation, and that treatment-induced changes are conditional on the combination of the two. Pain may alter opioid activity in a proinflammatory direction, or opioids may contribute to the proinflammatory effects of pain. It is possible that the independent effects of pain and opioids were small enough so as to be undetected with our measures, yet together reached a critical threshold for response. Impossible to determine is the relative contribution of opioids and pain on NF-κB activation, if at all. Further translational work is needed to confirm and characterize proinflammatory responses when pain and opioids are combined in the clinical setting and in relation to patient outcomes.

Limitations

Certainly, several limitations of the study temper interpretation of the findings. Most importantly, the design was observational, thus control treatments were absent and subjects were nonblinded to session, incurring the risk of placebo effect. Only one pain induction technique (CPT) was used; heat or electrical experimental pain stimuli might have induced more severe pain and a greater stress response. In vitro work suggests that other opioids (ie, morphine) may have a greater effect on certain proinflammatory markers than fentanyl, thus a direct empirical comparison of challenge opioids on proinflammatory response is needed. The order of combined treatments (opioid prior to pain) was fixed, and it is not known how responses might have appeared under conditions of pain preceding opioid administration. The sample size was small, and therefore interpretation of inferential analyses is necessarily limited. Although interindividual variation was controlled to the degree possible via the study protocol and data analysis techniques, Reyes-Gibby et al. provide evidence that in humans, cytokine gene polymorphisms of TNF-α and IL-6 exist which predict both pain severity and morphine analgesia. Further, the literature suggests that immune responses differ depending on whether opioid administration is acute or chronic, therefore findings cannot be generalized to patients who report ongoing opioid use. Finally, although providing insight into early events following experimental acute pain and opioids, a longer observation period may have revealed different patterns of inflammatory transcription factor expression; in all cases, sampling ended before NF-κB values had returned to baseline.
CONCLUSIONS

Several conclusions can be drawn from this study. First, in contrast to other reports with varying experimental approaches, neither slight experimental CPT pain nor opioid administration alone resulted in significant changes in NF-κB activation. This was true in stimulated and unstimulated immune cells, with patterns of response to these stimuli similar to those in the control conditions. However, in the healthy stimulated immune system, acute fentanyl given in the context of acute, relatively mild pain consistently and significantly increased activity in this inflammatory transcription system. This novel observation of increased activation of a proinflammatory transcription factor in the clinically relevant situation of acute pain plus opioid administration raises the intriguing possibility that this combination enhances the intended, short-term purposes of inflammation, namely, pathogen clearance and initiation of tissue repair and healing.

By mitigating the consequences of unrelieved pain, opioid analgesia has been associated with good health outcomes. A mechanism by which opioids in the presence of pain promote good outcomes may be by bolstering tissue healing systems. To the degree that inflammation is requisite of tissue healing, providing opioids to the patient with acute pain may facilitate innate immunity processes.

Acknowledgments

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References


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Figure 1.
Levels of intranuclear activated NF-κB induced in stimulated cells. Mean ± SD of increases in mean fluorescent intensity (MFI) compared to unstimulated cells (MFI delta = MFI stimulated cells – MFI unstimulated cells), adjusted for baseline MFI delta values, in (a) peripheral blood mononuclear cells (PBMC). Pain vs opioid + pain, p = 0.034. Opioid vs opioid + pain, p = 0.016. (b) Monocytes. Pain vs opioid + pain, p = 0.044. Opioid vs opioid + pain, p = 0.004. (c) Lymphocytes. Pain vs opioid + pain, p = 0.034. Opioid vs opioid + pain, p = 0.018. Control vs opioid + pain, p = 0.045. p values shown are for the indicated pairwise comparison by ANOVA across time points.
Table 1

Session<sup>a</sup> timeline and measures

<table>
<thead>
<tr>
<th>Measures</th>
<th>Fentanyl&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>CPT</th>
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<tbody>
<tr>
<td></td>
<td>~30 min baseline</td>
<td>~15 min</td>
</tr>
<tr>
<td>Pain&lt;sup&gt;‡&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold-pressor pain threshold</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Cold-pressor pain tolerance</td>
<td>X</td>
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</tr>
<tr>
<td>VAS pain severity</td>
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<td></td>
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<tr>
<td>VAS pain stress</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Opioid adjective checklist&lt;sup&gt;†&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF-κB</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

CPT, cold-pressor test; NF-κB, nuclear factor κB; VAS, visual analogue scale.

<sup>a</sup>One session per day, randomly scheduled, at least 48 h apart.

<sup>‡</sup>Sessions opioid and opioid + pain.

<sup>†</sup>Sessions pain and opioid + pain only.
### Table 2
Demographic characteristics of the study subjects (n = 21)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
<th>N (percent)</th>
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</thead>
<tbody>
<tr>
<td>Age, y</td>
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<tr>
<td>Years of education</td>
<td>15.7 ± 1.1</td>
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<td>Female</td>
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<tr>
<td>Marital status</td>
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<tr>
<td>Never married</td>
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<td>Married</td>
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<tr>
<td>Divorced</td>
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<tr>
<td>Other</td>
<td>4 (19.0)</td>
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<tr>
<td>Employment</td>
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<tr>
<td>Working fulltime</td>
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<tr>
<td>Working part time</td>
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<td>Unemployed</td>
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<td>In school</td>
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</tr>
<tr>
<td>Other</td>
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<td></td>
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<tr>
<td>Latin ethnicity</td>
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<td>Race</td>
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<tr>
<td>White</td>
<td>13 (61.9)</td>
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<tr>
<td>Black</td>
<td>2 (9.5)</td>
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<td>Multiple</td>
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Table 3
Mean (±SD) sympathetic responses to cold-pressor pain (n = 21)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Baseline CPT</th>
<th>Post-CPT</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>117 ± 12</td>
<td>125 ± 15</td>
<td>0.008</td>
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<tr>
<td>Mean blood pressure</td>
<td>85 ± 15</td>
<td>93 ± 12</td>
<td>0.047</td>
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<tr>
<td>Respiratory rate</td>
<td>16.3 ± 1.0</td>
<td>17.9 ± 2.7</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Bold values show the significance levels (p-values). CPT, cold-pressor test.
### Table 4
Mean (±SD) cold-pressor pain responses: pain-only and opioid + pain sessions (n = 21)

<table>
<thead>
<tr>
<th>Cold-pressor responses</th>
<th>Pain-only</th>
<th>Opioids + pain</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS pain stress rating (0–10)</td>
<td>1.2 ± 5.7</td>
<td>0.7 ± 2.1</td>
<td>0.316</td>
</tr>
<tr>
<td>Cold-pressor threshold, s</td>
<td>0.8 ± 3.9</td>
<td>0.6 ± 1.5</td>
<td>0.466</td>
</tr>
<tr>
<td>Cold-pressor tolerance, s</td>
<td>12.3 ± 7.3</td>
<td>19.2 ± 13.0</td>
<td>0.025</td>
</tr>
<tr>
<td>VAS pain severity rating (0–10)</td>
<td>95.6 ± 96.8</td>
<td>157.7 ± 106.8*</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Includes seven of 21 subjects with tolerance = 300 s.

Bold values show the significance levels (p-values). VAS, visual analogue scale.