Distinct Mechanisms Underlying Pronociceptive Effects of Opioids

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In addition to analgesia, opioids may also produce paradoxical pain amplification [opioid-induced hyperalgesia (OIH)] either on abrupt withdrawal or during continuous long-term application. Here, we assessed antinociceptive and pronociceptive effects of three clinically used opioids at C-fiber synapses in the rat spinal dorsal horn in vivo. During 60 min of intravenous infusions of remifentanil (450 μg·kg⁻¹·h⁻¹), fentanyl (48 μg·kg⁻¹·h⁻¹), or morphine (14 mg·kg⁻¹·h⁻¹), C-fiber-evoked field potentials were depressed and paired-pulse ratios (PPR) were increased, indicating a presynaptic inhibition by all three opioids. After withdrawal, postsynaptic responses were enhanced substantially for the remaining of the recording periods of at least 3 h. Withdrawal from remifentanil led to long-term potentiation (LTP) of synaptic strength in C-fibers via activation of spinal μ-opioid receptors (MORs) and spinal NMDA receptors (NMDARs). Fentanyl and morphine caused an enhancement of synaptic transmission at C-fibers, which involved two distinct mechanisms: (1) an opioid withdrawal LTP that also required activation of spinal MORs and NMDARs and that was associated with a decrease in PPR suggestive of a presynaptic mechanism of its expression, and (2) an immediate-onset, descending facilitation of C-fiber-evoked field potentials during and after intravenous infusion of fentanyl and morphine. Immediate-onset, descending facilitation was mediated by the activation of extraspinal MORs, descending serotonergic pathways, and spinal 5-hydroxytryptamine-3 receptors (5-HT₃Rs). Our study identified fundamentally different pronociceptive effects of clinically used opioids and suggests that OIH can be prevented by the combined use of NMDAR and 5-HT₃R antagonists.

Introduction

Opioids represent the gold standard for the treatment of acute and chronic pain. Opioids may, however, also cause paradoxical pain amplification (hyperalgesia) that develops either on their abrupt withdrawal or during their continuous long-term use. Opioid-induced hyperalgesia (OIH) has been reported in animal models and was confirmed in human subjects (Angst and Clark, 2006). We have recently discovered a spinal mechanism that likely contributes to OIH: synaptic long-term potentiation (LTP) after opioid withdrawal. When the ultra-short-acting μ-opioid receptor (MOR) agonist remifentanil is withdrawn abruptly in vivo, synaptic transmission between nociceptive C-fibers and neurons in superficial spinal dorsal horn is persistently potentiated (Drdla et al., 2009). Opioid withdrawal LTP requires activation of spinal MORs and spinal NMDA receptors (NMDARs). LTP at C-fiber synapses can also be induced by various types of noxious stimulation and constitutes a cellular model of hyperalgesia (Sandkühler, 2009).

It is presently unknown whether opioid withdrawal LTP also applies to other clinically used MOR agonists that differ erably with respect to their pharmacological profile. For example, remifentanil has an ultra-short half-life of several minutes (Egan et al., 1993) because of its rapid metabolism by blood and tissue esterases (Feldman et al., 1991). Most clinically used opioids, including fentanyl and morphine, have half-lives in the range of hours (Trescot et al., 2008). Remifentanil and fentanyl but not morphine cause considerable internalization of MORs (Trafton et al., 2000; Zaki et al., 2000). Furthermore, morphine has a broader opioid receptor binding profile (Matthes et al., 1998) compared with fentanyl and remifentanil.

Clinically used opioids all bind to MORs that are present pre-synaptically on spinal terminals of nociceptive fibers and post-synaptically on spinal dorsal horn neurons. Acute opioid application depresses transmitter release from central terminals of nociceptive fibers (Kohno et al., 1999; Heinke et al., 2011). This mechanism correlates to their analgesic effect. In contrast, postsynaptic G-protein coupling, activation of postsynaptic NMDARs and postsynaptic Ca²⁺ rise are required for the induction of opioid withdrawal LTP at C-fiber synapses (Drdla et al., 2009). It is presently not clear whether the expression of withdrawal LTP is also postsynaptic or whether it involves any pre-synaptic mechanisms.

MORs are also expressed on neurons in various brain areas, including the rostral ventromedial medulla, that send descending projections to the spinal cord (Marinelli et al., 2002). MOR agonists may activate descending pathways that modulate spinal nociception. Several lines of evidence suggest that descending facilitation contributes to OIH (Ossipov et al., 2004). Accordingly, surgical lesion (Vanderah et al., 2001) or pharmacological blockade of descending facil-
iation by intrathecal injection of a 5-hydroxytryptamine-3 receptor
(5-HT₃R) antagonist (Vera-Portocarrero et al., 2007; Liang et al.,
2011) prevents development of OIH. The spinal mechanisms of
opioid-induced descending facilitation of nociception are, however,
primarily unidentified.

The present study revealed that remifentanil, fentanyl, and mor-
phine all enhance synaptic transmission at spinal C-fibers but via
fundamentally different mechanisms. The expression of withdrawal
LTP by fentanyl and morphine but not remifentanil may involve a
presynaptic mechanism and was additionally boosted by ascending
descending facilitation via activation of spinal 5-HT₃Rs.

Materials and Methods

Animals. All procedures were in accordance with European Commu-

nities Council directives (86/609/EEC) and were approved by the Austrian
Federal Ministry of Science and Research.

Male Sprague Dawley rats (Medical University of Vienna breeding
facility) weighing between 150 and 250 g were used for all experiments.
Animals were kept on a 12 h light/dark cycle, housed three to six per cage,
and were provided food and water ad libitum.

Animal surgery in vivo. Isoflurane (4 vol%/min.) in two-thirds N₂O and
one-third O₂ was initially administered via a mask to induce anesthesia.
Animals were intubated using a 16 gauge cannula and then mechanically
ventilated at a rate of 75 strokes/min using a tidal volume of 4–6 ml.
Anesthesia was maintained by 1.5 vol%/min isoflurane. Body core tempera-
ture was kept at 37.5°C with a feedback-controlled heating blanket. Deep
surgical level of anesthesia was verified by stable mean arterial blood
pressure during noxious stimulation. Surgical procedures were per-
formed as described previously (Ikeda et al., 2006). Briefly, a jugular vein
and a carotid artery were cannulated to allow intraarterial infusions and
arterial blood pressure monitoring, respectively. Muscle relaxation was
achieved by 2 µg kg⁻¹ h⁻¹ intravenous pancuronium bromide. After
cannulation, the left sciatic nerve was dissected free for bipolar electrical
stimulation with a silver hook electrode. The lumbar segments L4 and L5
were exposed by laminectomy. The dura mater was carefully incised and
retracted. Two metal clamps were used for fixation of the vertebral col-
umn in a stereotactic frame. An agarose pool was formed around the
exposed spinal segments. The spinal cord was continuously superfused
with 5 ml of artificial CSF in which additional drugs could be dissolved as
indicated. At the end of each electrophysiological experiment, animals
were decapitated under deep anesthesia. The spinal cord was removed
and cryofixed for detection of a rhodamine B spot at the recording site
under a fluorescence microscope. Only those experiments in which the
recording site was located in laminae I or II were analyzed.

Drugs and drug administration. For in vivo recordings, pancuronium
bromide (Pancuronium-ratiopharm; Ratiopharm) was administered as
an intravenous infusion (2 µg kg⁻¹ h⁻¹). Remifentanil (Ultiva; kindly
provided by GlaxoSmithKline) was dissolved in sterile NaCl and applied
as a 30 µg/kg bolus injection, followed by a 1 h infusion at a rate of 450
µg kg⁻¹ h⁻¹. Fentanyl dihydrogenate citrate (Fentanyl-Janssen; Janssen-
Cilag Pharma) was applied as a 40 µg/kg bolus injection, followed by a 1 h
infusion at a rate of 48 µg kg⁻¹ h⁻¹. Morphine hydrochloride (Vendal;
Lannacher) was applied as an 8 mg/kg bolus injection, followed by an
infusion at a rate of 14 mg kg⁻¹ h⁻¹ for 1 h. Naloxone (Tocris Biosci-
ence) was dissolved in sterile NaCl before given intravenously (100
mg kg⁻¹ h⁻¹).

All other drugs were purchased from Tocris Bioscience. They were
dissolved in water and added directly to 5 ml of artificial CSF superfusate
to obtain the desired concentration as indicated: the MOR antagonist
α-Phe-Cys-Tyr-α-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP; 10 µM), the
competitive NMDAR antagonist d(-)-2-amino-5-phosphono pentanoic acid (D-
AP-5; 100 µM), the opioid receptor antagonist naloxone hydrochloride
(100 µM), and the 5-HT₃R antagonists granisetron hydrochloride (1 µM)
and ondansetron hydrochloride (1 µM).

Electrophysiological recording. Electrophysiological recordings were
performed as described previously (Ikeda et al., 2006). Briefly, C-fiber-
evoked field potentials were recorded with glass electrodes (impedance of
2–3 MΩ) from laminae I and II of the spinal cord dorsal horn in response
to stimulation of sciatic nerve fibers. The pipette solution consisted of
135 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 10 mM HEPES, 1 mM MgCl₂,
and 0.2% rhodamine B. At the end of each electrophysiological experi-
ment, the recording site was labeled by pressure application (300 mbar
for 1 min) with 0.2% rhodamine B via the electrode. Electrodes were
driven by a microstepping motor. Recordings were made with an ISO-
DAM-amplifier (World Precision Instruments) using a bandwidth filter
of 0.1–1000 Hz.Signals were monitored on a digital oscilloscope and
digitized by an analog-to-digital converter. Affluent input from the hind-
paw was identified by mechanical stimulation of the foot while acous-
tically evaluating the evoked responses with an audio monitor. Test stimuli
were delivered to the sciatic nerve and consisted of pulses of 0.5 ms
duration at 25 V applied every 5 min using an electrical stimulator (ISO-
01D-100; NPI Electronic). For paired-pulse recordings, two consecutive
stimuli were applied at a 500 ms interval.

Behavioral tests. Behavioral experiments were performed between 9:00
AM. and 6:00 P.M. Animals were habituated to the facility for at least 3 d
and handled by the experimenters during this time. For 2 d before the
assessment of baseline thresholds, rats were habituated to the behavioral
testing apparatus for 30 min. Mechanical thresholds were measured with
a calibrated von Frey monofilaments with incremental stiffness between
0.25 and 15 g (Stoelting) based on the up and down method of Dixon
(1965) at regular intervals. Rats were placed in individual Plexiglas boxes
on a wire mesh metal floor. The plantar surface of the hindpaw between
the footpads was stimulated in a consistent manner for 10 s. A foot
withdrawal not attributable to normal locomotion was counted as a posi-
tive response. A lower force hair was presented during a positive re-
sponse and a higher force during a negative response. A 50% threshold in
grams was calculated as described previously (Chaplan et al., 1994).
Experiments were performed by an experimenter unaware of treatment
groups. The response thresholds for each hindpaw were averaged.

Baseline threshold testing was initiated 3 h before the treatment. An-
esthesia was induced and maintained as described above for the in vivo
electrophysiology. Animals were intubated and mechanically ventilated.
The same drugs and doses were administered for 1 h into the jugular vein
as described above. For remifentanil, a saline infusion with the equivalent
concentration of glycine (3.4 mg kg⁻¹ h⁻¹ infusion) contained in Ultiva
(Hahnenkamp et al., 2004), whereas for fentanyl a physiological saline
infusion served as control. Cannulation was removed and the skin was
sutured using sterile precautions. Animals with remifentanil or respec-
tive control were removed from artificial ventilation and anesthesia 15
min after withdrawal. Anesthesia and ventilation of fentanyl-treated an-
imals or respective controls was terminated 1 h after the end of the
infusion, when spontaneous breathing restarted. Behavioral testing was
performed 4 h, 24 h, 72 h, and 7 d after withdrawal from opioids.

Statistical analyses. Data were analyzed using SigmaStat 3.1 (Systat
Software). For electrophysiological recordings, the area under the curve
of C-fiber-evoked field potentials was determined offline using Clampfit
10 (Molecular Devices). The mean area under the curve of six consecu-
tive stable field potentials before opioid application served as a baseline
control. Responses were normalized to the baseline in every animal. Data
were tested for normality using the Kruskal–Wallis test. Unless otherwise
indicated, a one-way repeated-measures (RM) ANOVA was performed
to compare the different experimental protocols and treatments.
Non-parametric one-way RM-ANOVA on ranks was performed in the case
of non-normality. Effects of treatments were assessed using a t test compar-
ing final size of potentiation from treated and nontreated animals. In case
normality failed, a Mann–Whitney U test was performed.

For analysis of the paired-pulse measurements, a PPR was calculated
dividing the area of the C-fiber-evoked field potential of the second
response by the area of the first response. One-way RM-ANOVA was
performed on the mean of six PPRs from the baseline, 15 min after the
start of the opioid infusion, and 15 min after withdrawal.

Behavioral data were analyzed by using a two-way RM-ANOVA com-
paring treatments and time points.

One-way RM-ANOVA was performed using Bonferroni’s adjust-
ment, and one-way RM-ANOVA on ranks was corrected by Dunnett’s
test. A p value of <0.001 was considered as statistically “highly signifi-
significant” and a p value of <0.05 as “significant.” Values are expressed as mean ± SEM.

Results
Fentanyl and morphine enhance synaptic transmission during precipitated and unprecipitated withdrawal
Withdrawal from an intravenous infusion of remifentanil was followed by a persistent enhancement of C-fiber-evoked field potentials (to 214 ± 26% of control at 220–240 min, p < 0.001, n = 8; Fig. 1A). Our previous study revealed that this enhancement is attributable to synaptic LTP, which is triggered by withdrawal from the opioid and which we thus termed “opioid withdrawal LTP” (Drdla et al., 2009). We showed that abrupt termination of a remifentanil infusion, which leads to a fast recovery from opioid-induced depression, is crucial for remifentanil-induced withdrawal LTP. A tapered withdrawal regimen, however, fully prevents LTP induction. Recovery from depression is slower for fentanyl and morphine because of their longer half-life. For quick recovery from depression, we precipitated the withdrawal by spinal application of the specific MOR antagonist CTOP at the end of the opioid infusion. During precipitated withdrawal, the recovery from depression was as fast as that observed after remifentanil. Under these conditions, fentanyl and morphine also elicited a robust enhancement of C-fiber-evoked field potentials (to 381 ± 57% of control at 220–240 min, p < 0.001, n = 7, respectively; Fig. 1B,C). This enhancement of C-fiber-evoked field potentials induced by fentanyl and morphine was attributable to two distinct mechanisms: (1) an opioid withdrawal LTP that required activation of spinal MORs and spinal NMDARs and (2) an immediate-onset, descending facilitation that was induced by activation of extraspinal MORs (see below). We collectively named both effects “opioid-induced enhancement of synaptic transmission.”

We next tested whether precipitation of the withdrawal was necessary for the induction of opioid-induced enhancement of synaptic transmission. Fentanyl and morphine were thus withdrawn without application of the MOR antagonist. The magnitude of C-fiber-evoked field potentials reached predrug level within 30 min after stopping the fentanyl infusion. It was followed by an enhancement of synaptic transmission that lasted until the end of the recording period (to 227 ± 44% of control at 280–300 min, p = 0.006, n = 8; Fig. 1D). After termination of the morphine infusion, the baseline was reached within 2 h and was followed by an enhancement of synaptic transmission (to 215 ± 50% of control at 440–460 min, p = 0.049, n = 6; Fig. 1E).

These results demonstrate that precipitated withdrawal or fast recovery from depression was not essential for the enhancement of synaptic transmission by fentanyl or morphine.

Presynaptic expression of the enhancement of synaptic transmission by fentanyl or morphine but not by remifentanil
The induction of withdrawal LTP by the MOR-specific agonist [d-Ala2,N-Me-Phe4,Gly-ol]-enkephalin (DAMGO) in vitro requires postsynaptic signaling (Drdla et al., 2009). Here, we tested whether the expression of opioid-induced enhancement of synaptic transmission is presynaptic and/or postsynaptic in nature. We evaluated the PPR of field potentials evoked by C-fiber stimulation before, during, and after administration of remifentanil, fentanyl, and morphine. PPR increased during remifentanil infusion (from 0.82 ± 0.03 during baseline to 1.15 ± 0.4 during infusion; p = 0.011, n = 11), consistent with a presynaptic inhi-
Intravenous opioids bidirectionally modulated the PPR of spinal C-fiber-evoked field potentials. Bar graphs represent the mean PPR during baseline (30 min before opioid infusion), during opioid infusion (at 15–45 min after onset of the infusion), and after withdrawal (at 15–45 min after termination of the infusion). A, During intravenous remifentanil infusion (dosing as in Fig. 1A), PPR was significantly increased and returned to baseline level after withdrawal. Fentanyl infusion (dosing as in Fig. 1B) was associated with an increased PPR. After withdrawal, precipitated by topical application of CTOP (10 μM), the PPR was depressed below baseline level. C, During morphine infusion (dosing as in Fig. 1C), the PPR increased significantly. After CTOP precipitated withdrawal, the PPR was decreased below baseline. *p < 0.05, **p < 0.01, ***p < 0.001, significant differences from baseline.

These results suggest that all three opioids depress C-fiber-evoked transmitter release presynaptically during their application. After withdrawal, fentanyl and morphine but not remifentanil may potentiate the transmitter release from C-fibers as a mechanism that enhances synaptic transmission.

Enhancement of synaptic transmission induced by fentanyl and morphine is partially independent of spinal NMDAR activation

Withdrawal LTP induced by remifentanil requires activation of spinal NMDARs (Drdla et al., 2009). In the present study, blockade of spinal NMDARs by topical application of D-AP-5 significantly reduced (p = 0.048) but failed to abolish enhancement of synaptic transmission after precipitated withdrawal from fentanyl (to 171 ± 29% of control at 220–240 min, p = 0.022, n = 6; Fig. 3A). Similarly, spinal D-AP-5 reduced (p = 0.004) but did not fully prevent enhancement of synaptic transmission after precipitated withdrawal from morphine (to 185 ± 23% of control at 220–240 min, p = 0.008, n = 6; Fig. 3B).

These results suggest that, in contrast to remifentanil, fentanyl and morphine may not only trigger opioid withdrawal LTP but also an additional mechanism that is independent of spinal NMDAR activation.

Immediate-onset facilitation of C-fiber-evoked potentials during systemic fentanyl and morphine but not remifentanil infusion

We next studied the role of spinal MORs and applied the specific MOR antagonist CTOP directly onto the spinal cord at the recording site throughout the recording period. This abolished both the depression of C-fiber-evoked field potentials by systemically applied remifentanil and the induction of withdrawal LTP.
Fentanyl but not remifentanil activates immediate-onset, descending facilitation via spinal 5-HT₃Rs

It has been shown that descending pathways (King et al., 2005) and activation of spinal 5-HT₃Rs (Vera-Portocarrero et al., 2007; Liang et al., 2011) are involved in OIH. To evaluate the contribution of descending serotonergic pathways to the immediate-onset facilitation induced by fentanyl, we superfused the spinal cord with the 5-HT₃R antagonists ondansetron or granisetron in the presence of spinal CTOP. Fentanyl-induced immediate-onset facilitation was fully blocked by ondansetron (113 ± 19% of control at 220 – 240 min, p < 0.001; data not shown) and by granisetron (107 ± 16% of control at 220 – 240 min, p = 0.563, n = 7; Fig. 5B). Thus, activation of descending serotonergic pathways and spinal 5-HT₃Rs is essential for fentanyl-induced immediate-onset facilitation. We next asked whether the immediate-onset, descending facilitation interacts with the withdrawal of fentanyl, and morphine infusion when spinal MORs were blocked with CTOP.
drawal LTP. We thus induced a precipitated withdrawal from fentanyl in the presence of spinal granisetron to block descending facilitation. At least two scenarios are conceivable. First, the spinal mechanisms of withdrawal LTP and immediate-onset facilitation could overlap and thus occlude each other. In this case, both effects should be sub-additive when leading to opioid-induced enhancement of synaptic transmission. Under spinal 5-HT<sub>3</sub>R blockade, PPR was still depressed after withdrawal from intravenous fentanyl (from 0.75 ± 0.19 during baseline to 0.43 ± 0.06 after withdrawal, p = 0.023, n = 6; data not shown). Thus, 5-HT<sub>3</sub>R activation is not required for PPR depression induced by withdrawal of fentanyl. Together, these findings indicate that immediate-onset, descending facilitation and opioid withdrawal LTP are independent pronociceptive mechanisms of opioids.

Spinal granisetron did not affect (p = 0.513) remifentanil-induced withdrawal LTP (to 192 ± 19% of control at 220–240 min, p < 0.001, n = 8; data not shown), supporting that immediate-onset, descending facilitation is not activated by remifentanil.

Complete block of spinal pronociceptive effects of fentanyl and morphine
We next tested whether withdrawal LTP and immediate-onset, descending facilitation by fentanyl or morphine can be blocked fully without compromising inhibition. We simultaneously superfused the spinal cord with the NMDAR antagonist D-AP-5 to block withdrawal LTP and the 5-HT<sub>3</sub>R antagonist granisetron to block immediate-onset, descending facilitation. This fully prevented the rise of C-fiber-evoked field potentials after fentanyl (105 ± 15% of control at 220–240 min, p = 1.00, n = 6; Fig. 6A). The fentanyl-induced depression was, in contrast, fully preserved. Similar results were obtained for morphine (112 ± 19% of control at 220–240 min, p = 0.749, n = 7; Fig. 6B).

Thus, the two pronociceptive mechanisms that are activated by fentanyl and morphine, i.e., the NMDAR-dependent withdrawal LTP, and the 5-HT<sub>3</sub>R-mediated immediate-onset, de-
Opioid-induced depression at spinal C-fiber synapses

In the superficial spinal dorsal horn, MORs are present on the terminals of afferent nerve fibers, including C-fibers, and postsynaptically on dorsal horn neurons (Besse et al., 1990; Scherrer et al., 2009). We and others have shown previously that, in vitro, the MOR agonist DAMGO induces a powerful presynaptic depression at C-fiber terminals (Iokma et al., 2007; Heinke et al., 2011). Similarly, in the present study, depression of C-fiber-evoked field potentials by systemic remifentanil, fentanyl, and morphine required activation of spinal MORs and was associated with an increase in the PPR. The PPR is an indication to assess changes in neurotransmitter release probability in patch clamp as well as in vivo field potential recordings (Zucker, 1973; Zucker and Regenhr, 2002). Collectively, these data suggest that, after systemic application, the three opioids act on spinal MORs to depress neurotransmitter release from nociceptive C-fibers as a mechanism of antinocepcion.

Enhancement of synaptic transmission by remifentanil, fentanyl, and morphine

After withdrawal, all three opioids induced a robust enhancement of C-fiber-evoked field potentials. Compared with remifentanil, recovery from depression takes considerably longer for fentanyl and morphine because of their longer half-life. Our previous study revealed that LTP during abrupt withdrawal from remifentanil is fully prevented when a tapered withdrawal regimen is used, which leads to a slower recovery from depression (Drda et al., 2009). The slower recovery from depression by morphine or fentanyl without precipitation was comparable with the recovery rate after tapered withdrawal from remifentanil. This did, however, not prevent opioid-induced enhancement of synaptic transmission and suggests that the rate of recovery from depression is not a determinant parameter for enhancement of synaptic transmission by fentanyl and morphine.

Enhancement of synaptic transmission by fentanyl was significantly reduced by the blockade of spinal NMDARs as well as by the blockade of spinal 5-HT3Rs. However, only simultaneous blockade of both receptors could fully prevent opioid-induced enhancement of synaptic transmission, indicating that two distinct mechanisms mediate this effect: opioid withdrawal LTP and immediate-onset, descending facilitation.

Withdrawal LTP by remifentanil, fentanyl, and morphine

The withdrawal LTP induced by the three opioids differed with respect to their impact on the PPR. In contrast to remifentanil, the expression of morphine- and fentanyl-induced withdrawal LTP was associated with a decreased PPR, suggesting an enhanced transmitter release probability (Oleskevich et al., 2000; Thomson, 2000). When spinal MORs were blocked by CTOP, fentanyl and morphine induced neither a paired-pulse facilitation nor a paired-pulse depression. This indicates that spinal MORs mediate both phenomena. Our results are in contrast to those of Zhou et al. (2010) who reported that the MOR agonist DAMGO enhanced PPR, during both acute synaptic depression and withdrawal LTP in a spinal cord slice preparation. Surprisingly, these authors interpreted the increased PPR as a sign for presynaptic expression of LTP (see also our eLetter in response to their report at http://www.jneurosci.org/content/30/12/4460.long/reply#jneuro_el_71584). Nevertheless, the data sug-
gest that different opioids can have diverging effects on the PPR at C-fiber synapses. We have shown previously that postsynaptic sig-
naling is essential for the induction of withdrawal LTP (Drdla et al.,
2009). In the present work, we extended these findings by showing
that enhanced transmitter release likely contributes to the expression
of withdrawal LTP induced by fentanyl and morphine but not by
remifentanil at C-fiber synapses.

We suggest that opioid withdrawal LTP underlies OIH be-
cause both can be induced by identical dosing regimen and in-
volve overlapping signaling pathways, including activation of
NMDARs (Trujillo and Akil, 1991; Célèrier et al., 1999) and pro-
tein kinase C (Mao et al., 1994; Sweitzer et al., 2004; Drdla et al.,
2009). Fentanyl and morphine but not remifentanil activate in
addition serotonergic descending facilitatory pathways, which
may further boost OIH.

Immediate-onset, descending facilitation induced by
morphine and fentanyl but not by remifentanil
Several days after continuous application of opioids, the initial
analgesia may not only vanish but may actually turn into OIH
(Ossipov et al., 2004; King et al., 2005). OIH may involve activa-
tion of descending serotonergic facilitatory systems because it can
be blocked by surgical disruption of descending pathways (Van-
derah et al., 2001) or by blocking spinal 5-HT3Rs (Vera-
Portocarrero et al., 2007; Liang et al., 2011). The present study
identified a novel, immediate-onset, descending facilitation of
synaptic strength in C-fibers activated by opioids. This process
could also well underlie the slowly developing loss of analgesic
efficacy (apparent tolerance) and OIH. Within minutes of sys-
temic application, both fentanyl and morphine triggered a facilita-
tion that progressively increased during the application and
continued to increase throughout the recording period after ter-
mination of the opioid infusion. This immediate-onset, descend-
ing facilitation by morphine and fentanyl was normally masked
by the concomitant depression of spinal nociception but became
apparent when spinal MORs were blocked.

Systemic but not spinal opioid-receptor blockade abolished
immediate-onset, descending facilitation induced by intravenous
infusion of fentanyl, demonstrating the involvement of extraspinal
opioid receptors. Good candidates are MORs in the rostral
ventromedial medulla, a brainstem region that sends serotoner-
gic pathways to the spinal dorsal horn (Fields and Basbaum,
2000) and that has been implicated in the expression of OIH
(Porreca et al., 2002). Descending serotonergic pathways can be
activated by ascending pathways involving neurokinin 1 recep-
tor-positive projection neurons (Suzuki et al., 2002). Previ-
ous studies using combined electrophysiological and immuno-
histological approaches indicate that serotonergic descending
pathways can also be disinhibited by MOR agonists (Marinelli et
al., 2002). Disinhibition has a potentially rapid onset that could
correspond well to the time course of the presently described
immediate-onset facilitation.

Immediate-onset, descending facilitation identified in the
present study required activation of spinal 5-HT3Rs, and it is safe
to say that it involves descending serotonergic pathways because
they are the only relevant source of serotonin in the spinal dorsal
horn (Millan, 2002). Some studies propose that 5-HT3Rs activation
enhances transmitter release from presynaptic terminals of
afferent fibers (Nayak et al., 1999; Suzuki et al., 2004). The
immediate-onset, descending facilitation shown in the present
study was, however, not associated with any change in the PPR.
This suggests that 5-HT3R activation during descending facilita-
tion did not enhance transmitter release from presynaptic termi-
nals but could have rather affected superficial spinal dorsal
neurons expressing the 5-HT3Rs (Kia et al., 1995; Miquel et al.,
2002; Conte et al., 2005). 5-HT3Rs are not coupled to G-proteins
but directly to nonsselective cationic channels (Derkach et al.,
1989). Activation of 5-HT3Rs located on somadendritic regions
of spinal dorsal horn neurons induce an inward current that may
exert both pronociceptive and antinociceptive effects because
5-HT3Rs are expressed on spinal GABAergic and on putatively
excitatory interneurons (Fukushima et al., 2009).

Differential pronociceptive mechanisms activated by
remifentanil, fentanyl, and morphine
We have found major differences in the spinal pronociceptive
mechanisms of remifentanil on the one hand and fentanyl and
morphine on the other. The question arises which properties
might cause this grouping. Characteristics that are not shared by
fentanyl and morphine can be excluded, such as the low potency
to induce MOR internalization (Trafton et al., 2000; Zaki et al.,
2000), the broad opioid receptor subtype binding (Matthes et
al., 1998), and the production of active metabolites (Yaksh et al.,
1986), which are all typical for morphine but not for fentanyl.
Lipophilicity is also an unlikely grouping variable because fenta-
nyl but not morphine and remifentanil is characterized by a very
high lipophilicity. One distinguishing characteristic could be that
the three opioids do not act on the same sites in the CNS because
of an unequal ability to activate different splice variants of the
MOR gene (Pasternak, 2004). The relevance of this is, however,
presently unknown.

The present study revealed substantial differences in the
mechanisms that underlie the enhancement of spinal nocicep-
tion by three systemically applied opioids. Although remifentanil
selectively induced an NMDAR-dependent withdrawal LTP via
activation of spinal MORs, fentanyl and morphine in addition
activated descending, facilitatory, serotonergic pathways via ex-
traspineal MORs. Our findings add to the list of distinguishing
features between opioids and provide an additional rationale for
opioid rotation in pain patients. Our data further suggest that a
combination of NMDAR antagonists with opioids, such as fen-
tanyl or morphine, may reduce but may not fully prevent OIH.
An additional antagonist at the 5-HT3R may prove to be useful
(Liang et al., 2011; Vera-Portocarrero et al., 2011). Clinically used
5-HT3R antagonists are available for the treatment of emesis and
pruritus, e.g., caused by opioids, and include granisetron, dolas-
etron, ondansetron, palonosetron, and tropisetron. The present
findings provide another motivation for combining these drugs
with opioids from early on.

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