Erasure of a Spinal Memory Trace of Pain by a Brief, High-Dose Opioid Administration

Ruth Drdla-Schutting, Justus Benrather, Gabriele Wunderbaldinger, Jürgen Sandkühler

Painful stimuli activate nociceptive C fibers and induce synaptic long-term potentiation (LTP) at their spinal terminals. LTP at C-fiber synapses represents a cellular model for pain amplification (hyperalgesia) and for a memory trace of pain. μ-Opioid receptor agonists exert a powerful but reversible depression at C-fiber synapses that renders the continuous application of low opioid doses the gold standard in pain therapy. We discovered that brief application of a high opioid dose reversed various forms of activity-dependent LTP at C-fiber synapses. Depotentiation involved Ca2+-dependent signaling and normalization of the phosphorylation state of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. This also reversed hyperalgesia in behaving animals. Opioids thus not only temporarily dampen pain but may also erase a spinal memory trace of pain.

References and Notes
8. Z. Weinberg et al., Genome Biol. 11, R31 (2010).
9. Supporting text and materials and methods are available as supporting material on Science Online.

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Supporting Online Material
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Synaptic long-term potentiation (LTP) is a cellular model for learning and memory formation. The reversal of LTP, that is, synaptic depotentiation, is a potential mechanism of memory erasure (7). Depotentiation involves Ca$^{2+}$-dependent signaling (8) and may reverse LTP-associated changes in the phosphorylation state of α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid receptors (AMPARs) (9). We tested the hypothesis that brief application of a MOR agonist reverses LTP at C-fiber synapses in superficial lumbar dorsal horn by Ca$^{2+}$-dependent signaling pathways, which normalize the phosphorylation state of AMPAR subunits.

In adult rats, conditioning low-frequency stimulation (LFS) of sciatic nerve fibers at C-fiber intensity induced LTP of spinal C fiber–evoked field potentials (10) (Fig. 1A). Brief intravenous infusion of a high dose of the ultrashort-acting MOR agonist remifentanil (450 μg·kg$^{-1}$·hour$^{-1}$) acutely depressed potentiated responses. Upon termination of the remifentanil infusion, C fiber–evoked field potentials did not return to the elevated predrug levels but were significantly depotentiated from 188 ± 11% to 128 ± 14% of control values before LFS (n = 25, P < 0.001, Fig. 1B). This is in contrast to the potentiation of C fiber–evoked field potentials upon wash-out of remifentanil in naïve animals (3, 11) and thus cannot be explained by an incomplete wash-out of the opioid. A second application of remifentanil given 1 hour later fully reversed LTP (depotentiation from 180 ± 11% (mean ± SEM) to 128 ± 16% of control after the first application, P = 0.001; and to 76 ± 14% after second application, P < 0.001; n = 6, Fig. 1C). A lower dose of remifentanil (225 μg·kg$^{-1}$·hour$^{-1}$) was, however, ineffective (196 ± 22% versus 194 ± 12% of control, n = 5, P = 1, Fig. 1D).

Our previous study revealed that abrupt but not tapered withdrawal from remifentanil induces LTP at naïve synapses (3). Here, the same tapering regimen had, in contrast, no effect on the efficacy of opioid-induced depotentiation (OID; depotentiation from 180 ± 13% to 123 ± 10% of control, n = 6, P = 0.002; fig. S1A).

OID was unaffected by blockade of spinal γ-aminobutyric acid type A (GABA$\mathrm{A}$) receptors with picrotoxin (depotentiation from 216 ± 19% to 151 ± 22% of control, n = 7, P = 0.001; fig. S1B), indicating that an enhanced inhibition via GABA$\mathrm{A}$ receptors is not involved.

Acute depression and OID were fully blocked by intravenous application of the opioid receptor antagonist naloxone (fig. S1C) and by spinal application of the selective MOR antagonist t-Phe-

\[
\text{Cys-Tyr-}\text{o-Trp-Om-Thr-Pen-Thr-NH}_2
\]

(CTOP, Fig. 2A), demonstrating that activation of spinal MORs is essential for both effects.

Withdrawal from opioids may trigger the release of glutamate and the activation of Ca$^{2+}$-permeable glutamate receptors of the NMDA subtype (12). The activation of group I metabotropic glutamate receptors (group 1 mGluRs) may lead to an additional rise in free cytosolic Ca$^{2+}$ by Ca$^{2+}$ release from intracellular stores. Blockade of Ca$^{2+}$ entry through spinal NMDA receptors (Fig. 2B), blockade of group I mGluRs (Fig. 2C), or blockade of Ca$^{2+}$ release from ryanodine-sensitive intracellular stores with dantrolene (Fig. 2D) all abolished OID but not the acute depression by the opioid.

LTP at the first synaptic relay in nociceptive pathways requires activation of Ca$^{2+}$/calmodulin-dependent protein kinase II and protein kinase C (PKC) (13, 14), which phosphorylate the GluR1 subunit of the AMPA receptor at Ser$^{381}$ (15, 16). Interestingly, the time course of Ser$^{381}$ phosphorylation in spinal dorsal horn parallels post-injury pain amplification (17) and is located to the superficial spinal dorsal horn (18), where C fibers terminate. Here, LFS-induced LTP was also associated with changes in the phosphorylation state of AMPARs, and we speculated that OID may reverse these changes. LFS caused enhanced phosphorylation of surface GluR1 subunits of AMPARs at Ser$^{381}$ (S831-p; ratio of S831-p to total GluR1 protein levels was increased to 304 ± 60% of control, n = 12, P = 0.006). Remifentanil fully reversed this phosphorylation (ratio of S831-p to total GluR1 protein levels was 118 ± 21% of control, n = 12, P = 0.41 compared to control, P = 0.007 compared to LFS group; Fig. 3A). Dephosphorylation of spinal AMPARs at Ser$^{381}$, for example, by protein phosphatase 1 (PP1), reduces single-channel conductance and may thereby normalize synaptic strength (9). Accordingly, OID was completely prevented by blockade of PP1 (Fig. 2E).

LFS also induced a dephosphorylation of GluR2 subunits of AMPARs at Ser$^{880}$ (S880-p) in the spinal dorsal horn (ratio of S880-p to total GluR2 protein levels was reduced to 67 ± 10% of control, n = 12, P = 0.007; Fig. 3B). After the opioid application, Ser$^{880}$ was rephosphorylated (ratio of S880-p to total GluR2 protein levels was 142 ± 13% of control, n = 6, P = 0.004, Fig. 3C).

\[\text{Area of C fiber-evoked field potential (% of control)}\]

\[\text{Time (min)}\]

\[\text{Time (min)}\]

\[\text{Area of C fiber-evoked field potential (% of control)}\]

\[\text{Time (min)}\]

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\[\text{Time (min)}\]
Fig. 2. Signaling pathways involved in opioid-induced depotentiation. LTP induction and remifentanil application as in Fig. 1. Different blockers were applied directly onto the spinal cord at the recording segment (dashed horizontal bars). Insets show original traces of field potentials recorded at indicated time points; calibration bars, 50 ms and 0.2 mV. (A) Spinal superfusion with the MOR antagonist CTOP (10 μM) abolished acute depression and depotentiation in all animals tested (n = 5, P = 1). (B to F), OID was prevented by topical application of the NMDA receptor antagonist D-AP5 (100 μM, n = 5, P = 1), the mGluR antagonists MPEP (100 μM) and LY367385 (300 μM, n = 5, P = 0.6), the Ryanodine receptor blocker dantrolene (500 μM, n = 5, P = 1), the PP1 inhibitor calyculin A (300 nM, n = 5, P = 1), or the PKC blocker chelerythrine (800 μM, n = 7, P = 1). In all experiments, statistical significance was determined by using a one-way RM ANOVA.

Fig. 3. Quantitative analysis of phosphorylation of surface AMPAR subunits after LFS and opioid treatment. Bar graphs summarize the ratio of phospho-GluR1-Ser831 (S831-p) with total GluR1 (A) and ratio of phospho-GluR2-Ser880 (S880-p) with total GluR2 (B). Data are expressed as mean ± 1 SEM. Tissue samples were taken from naïve animals (Ctrl, n = 12), from animals 180 min post-LFS (LFS, n = 12), or from animals 180 min post-LFS with an additional 60-min remifentanil infusion (Remi, n = 12). Representative, corresponding Western blots are shown at the bottom. *P < 0.05. (A) LFS induced phosphorylation of AMPAR GluR1 subunits at Ser831, which was reversed by opioid treatment. (B) LFS dephosphorylated AMPAR GluR2 subunits at Ser880, whereas remifentanil induced rephosphorylation at this site.

was 127 ± 23% of control, n = 12, P = 0.028; Fig. 3B). PKC phosphorylates GluR2 subunit at Ser880. This reduces glutamatergic synaptic transmission by promoting receptor endocytosis (19). Consistently, blockade of PKC fully prevented OID without affecting acute depression (Fig. 2F). We found no evidence for significant changes in the phosphorylation state of GluR1 subunit of the AMPAR at Ser845 after LFS (ratio of S845-p to total GluR1 protein levels was 160 ± 38% of control, n = 12, P = 0.142) or after opioid application (93 ± 17% of control, n = 12, P = 0.706). The above described changes in the phosphorylation state of the AMPAR after LFS may enhance glutamatergic synaptic transmission (9), and their reversal may thus lead to OID.

The induction phase of spinal LTP, which lasts for 1 to 3 hours, involves posttranslational modifications, including changes in the phosphorylation state of synaptic proteins. The maintenance phase of LTP [≥3 hours in spinal cord (20) and brain (21)] may in addition also involve de novo protein synthesis. We thus asked whether LTP in the maintenance phase can also be depotentiated by opioids. When remifentanil was given 4 hours after LTP induction, OID was as effective (depotentiation from 193 ± 22% to 131 ± 14%, n = 10, P = 0.028; fig. S2, A and B) as when given after 1 hour. The reversal of late-phase LTP by remifentanil was, in contrast to that of early-phase LTP, not blocked by the PP1 inhibitor calyculin A (220 ± 22% versus 156 ± 20%, n = 10, P = 0.024; fig. S2C).

The persistently active protein kinase Mζ (PKMζ) is required in the spinal cord for maintaining tactile allodynia after intraplantar injection of interleukin-6 (22). We thus asked whether PKMζ in spinal cord also plays a role for the maintenance phase of LTP (22, 23) after LFS. PKMζ inhibitor ZIP had, however, no obvious
effect on the maintenance of LFS-induced LTP within the observation period of 6 hours (fig. S2D).

Depending on the type of conditioning stimulus, distinct forms of LTP are induced at C-fiber synapses, which affect different groups of postsynaptic neurons (13, 24) and involve signaling pathways that overlap only partially (13, 24, 25). We therefore tested whether OID can also be achieved for other forms of established spinal LTP. We induced LTP by conditioning high-frequency stimulation (HFS, 100 Hz; fig. S3A) of sciatic nerve fibers or by subcutaneous capsaicin injections (fig. S3C). The latter selectively activates nociceptive nerve fibers, which express the transient receptor potential channel subfamily V member 1 (TRPV1). Remifentanil also fully reversed these forms of LTP (after HFS, depotentiation was from 158% ± 8% to 99% ± 9%, n = 12, P < 0.001; after capsaicin, depotentiation was from 170% ± 16% to 100% ± 13%, n = 5, P < 0.001; fig. S3, B and D), demonstrating that OID applies to various forms of activity-dependent LTP at C-fiber synapses.

LTP is a synaptic model for some forms of hyperalgesia (26). We therefore asked whether OID has any relevance for behaving animals. Subcutaneous injections of capsaicin quickly led to mechanical hyperalgesia at the injected hindpaw (Fig. 4). The same dosage regimen of remifentanil that caused OID significantly attenuated capsaicin-induced hyperalgesia (Fig. 4A). Not surprisingly, the behavioral hyperalgesia was reversed only partially by the opioid treatment because additional peripheral and central mechanisms contribute to capsaicin-induced hyperalgesia (27, 28). PP1 inhibitor calyculin A fully blocked the attenuation of hyperalgesia by remifentanil (Fig. 4B), suggesting that depotentiation at nociceptive C-fibers may erase a memory trace of pain. LTP is expressed in ascending nociceptive pathways, which are relevant for the aversive components of pain. It will thus be interesting to explore whether opioids may also reverse the tonic-aversive state of pain (29).

Taken together, the present and our previous data demonstrate that activation of spinal MORs triggers distinct, bidirectional, and state-dependent synaptic plasticity in naïve versus potentiated C-fiber synapses. Remifentanil activates Ca2+-dependent signaling pathways, leading to activation of PP1 and PKC. At potentiated synapses, this normalizes the phosphorylation state of GluR1 at Ser^831 and that of GluR2 at Ser^880 and thereby depotentiates synaptic strength in C fibers. The presently identified reversal of synaptic LTP in nociceptive pathways provides a rationale for novel therapeutic strategies to cure rather than to temporarily dampen some forms of pain with opioids.

References and Notes
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10. Materials and methods are available as supporting online material at Science Online.
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Fig. 4. Capsaicin-induced mechanical hyperalgesia is reduced after opioid administration. Capsaicin injection (time point zero, arrow) significantly reduced mechanical withdrawal thresholds in the ipsilateral paw of awake, drug-free rats. (A) One group of animals received a 1-hour high-dose remifentanil infusion (horizontal bar) 60 min after capsaicin injection (blue circles, n = 7). A control group was treated with an intravenous saline infusion (red squares, n = 7). After wash-out of the opioid, mechanical thresholds were elevated significantly compared with thresholds before opioid treatment at 150 min (P = 0.05), 180 min (P = 0.003), 240 min (P = 0.004), and 300 min (P = 0.002), indicating partial reversal of hyperalgesia. (B) Opioid-induced reduction of mechanical hyperalgesia is blocked by the PP1 inhibitor calyculin A. After capsaicin injection, calyculin A (300 nM, 10 μl) was injected intrathecally 10 min before a 1-hour high-dose remifentanil infusion (red triangles, n = 7). Intrathecal injections of saline served as control (blue circles, n = 7). After wash-out of the opioid, mechanical thresholds were elevated significantly compared with thresholds before opioid treatment in the control group at 150 min (P = 0.021), 180 min (P = 0.019), 240 min (P = 0.003), and 300 min (P < 0.001). No effect of the opioid treatment on mechanical thresholds could be observed in the calyculin A–treated group (P = 0.523). One-way RM ANOVA or RM ANOVA on ranks was used for statistical comparisons.