Cellular/Molecular

Shared Mechanisms for Opioid Tolerance and a Transition to Chronic Pain

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Clinical pain conditions may remain responsive to opiate analgesics for extended periods, but such persistent acute pain can undergo a transition to an opiate-resistant chronic pain state that becomes a much more serious clinical problem. To test the hypothesis that cellular mechanisms of chronic pain in the primary afferent also contribute to the development of opiate resistance, we used a recently developed model of the transition of from acute to chronic pain, hyperalgesic priming. Repeated intradermal administration of the potent and highly selective μ-opioid agonist, [D-Ala^2,*N*-MePhe^4,gly-ol]-enkephalin (DAMGO), to produce tolerance for its inhibition of prostaglandin E_2 hyperalgesia, simultaneously produced hyperalgesic priming. Conversely, injection of an inflammogen, carrageenan, used to produce priming produced DAMGO tolerance. Both effects were prevented by inhibition of protein kinase Cε (PKCε). Carrageenan also induced opioid dependence, manifest as μ-opioid receptor antagonist (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2)-induced hyperalgesia that, like priming, was PKCε and G_i dependent. These findings suggest that the transition from acute to chronic pain, and development of μ-opioid receptor tolerance and dependence may be linked by common cellular mechanisms in the primary afferent.

Introduction

A common clinical observation is that a critical transition occurs in patients when opioid-sensitive "persistent acute pain" state transforms into an opioid-resistant "chronic pain" syndrome. Why analgesics are often less effective for the treatment of chronic than acute pain (Kalso et al., 2004; McCleane and Smith, 2007; Rosenblum et al., 2008) remains a critically important question, the answer to which could lead to improvement in the treatment of millions of patients with chronic pain syndromes.

We have developed a model of the transition to chronic pain, known as hyperalgesic priming (Aley et al., 2000), in which there is a long-lasting neuroplastic change in the signaling pathway mediating proinflammatory cytokine-induced nociceptor sensitization and mechanical hyperalgesia, at the site of a previous inflammatory insult (Reichling and Levine, 2009). Induction of hyperalgesic priming in the peripheral terminals of primary afferent nociceptors is mediated by protein kinase Cε (PKCε). The development of tolerance and dependence in μ-opioid receptor signaling also involves PKC (Mestek et al., 1995; Kelly et al., 2008) and switching between G_s- and G_i-mediated signaling pathways (Kalso et al., 2004; Chakrabarti et al., 2005; Wang and Burns, 2006; Rosenblum et al., 2008) that may also occur in sensory neurons (King et al., 1999). Therefore, we hypothesized that the reason chronic pain is associated with resistance to opiate analgesics is that both phenomena arise from closely related changes in intracellular signaling pathways in primary afferent nociceptors.

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Jon Levine is named on a patent for the use of PKCε in the treatment of pain.

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In the present study we tested this hypothesis by determining whether: 1) induction of opioid tolerance, by repeated administration of a μ-opioid agonist will also induce hyperalgesic priming, 2) induction of hyperalgesic priming by inflammation also induces opioid tolerance and dependence, and 3) interactions between the transition from acute to chronic pain and the development of opioid tolerance and dependence are mediated by PKCε and G_s/G_i switching in G-protein signaling in primary afferent nociceptors.

Materials and Methods

Animals. Experiments were performed on adult male Sprague Dawley rats (220–300 g; Charles River). Animals were housed 3 per cage, under a 12 h light/dark cycle, in a controlled environment at the University of California, San Francisco animal care facility. Food and water were available *ad libitum*. All testing was done between 10:00 A.M. and 4:00 P.M. Experimental protocols, approved by the University of California, San Francisco Committee on Animal Research, conformed to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Nociceptive testing. The nociceptive flexion reflex was quantified with a Basile Analgesymeter (Stoelting), which applies a linearly increasing mechanical force to the dorsum of a rat's hindpaw. Nociceptive threshold, defined as the force in grams at which the rat withdraws its paw, is the mean of 3 readings taken at 5 min intervals. For nociceptive testing, rats were placed in cylindrical transparent restrainers designed to provide adequate comfort and ventilation, allow extension of the hind leg from the cylinder, and minimize stress. All rats were acclimatized to the testing procedure. Each paw was treated as an independent measure and each experiment performed on a separate group of rats. The results are expressed as percentage change from baseline mechanical nociceptive threshold determined before administration of test agent.

Drugs and their administration. Drugs used in this study were prostaglandin E_2 (PGE_2; a hyperalgesic agent that directly sensitizes nociceptors), γ carrageenan (CARR, inflammogen) and pertussis toxin (PTX, a selective inhibitor of G_i-proteins) from Sigma; [D-Ala^2,*N*-MePhe^4,gly-ol]-enkephalin (DAMGO) (a μ-opioid receptor agonist) from Research

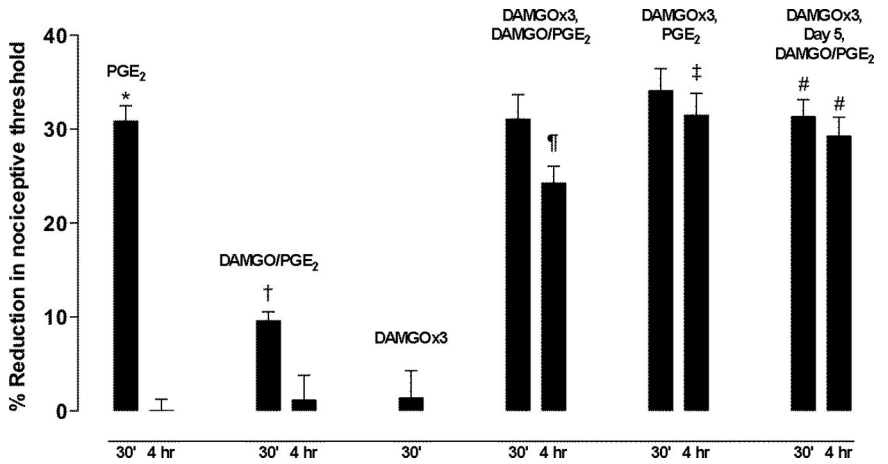

Figure 1. In naive control rats prostaglandin E_2 (PGE_2) 100 ng induces mechanical hyperalgesia (*$p < 0.001$ compared with baseline at 30 min (30′, $n = 12$) that lasts <4 h. When injected with PGE_2, μ-opioid receptor agonist DAMGO (1 μg) significantly inhibited PGE_2 hyperalgesia (DAMGO/PGE_2, †$p < 0.001$ compared with PGE_2 alone, $n = 6$). Following DAMGOx3 treatment, at the fourth hour, injected with PGE_2, DAMGO no longer inhibited PGE_2 hyperalgesia (DAMGOx3, DAMGO/PGE_2, p = NS, $n = 12$). However, unlike in the naive rats, PGE_2 hyperalgesia was still present at fourth hour in the DAMGOx3-pretreated rats (DAMGOx3, fourth hour DAMGO/PGE_2 ¶$p < 0.001$, $n = 12$). After three hourly injections of DAMGO, PGE_2 alone also produced prolonged (>4 h) hyperalgesia (DAMGOx3, PGE_2, ‡$p < 0.001$, $n = 6$). The tolerance to DAMGO inhibition of PGE_2 hyperalgesia and prolongation of PGE_2 hyperalgesia was still observed 5 d after 3 hourly doses of DAMGO (DAMGOx3, day 5, DAMGO/PGE_2, both #$p < 0.001$, $n = 6$). In all experiments, measurement of paw withdrawal threshold was done at 30 min (30′) and 4 h (4 h) after the administration of the test agents.

Biochemicals, pseudo receptor octapeptide for activated PKCε (ψεRACK; a specific agonist of PKCε) from SynPep Corp., D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP), a potent and highly selective μ-opioid receptor antagonist (Tocris Bioscience), and EAVSLKPT ($PKC\varepsilon_{V1-2}$, a selective PKCε translocation inhibitor peptide, EMD Bioscience). The selection of the drug doses used in this study was based on dose–response curves determined in previously published studies (Aley and Levine, 1997; Aley et al., 2000; Liu and Anand, 2001; Joseph and Levine, 2004; Joseph et al., 2004, 2008). The stock solution of PGE_2 (10 μg/μl) was prepared in ethanol, and further dilutions made in saline, yielding a final ethanol concentration of <1%. All other drugs were dissolved in saline. All drugs administered intradermally were in a volume of 5 μl using a 30-gauge hypodermic needle attached to a 10 μl Hamilton syringe, except carrageenan, which because of its high viscosity, was injected using a 27-gauge needle. When an antagonist was included, it was injected either 30 min before the agonist or coinjected with the agonist. When drug combinations were coinjected, they were administered from the same syringe so that the drug listed first, reached the intradermal site first.

Antisense and mismatch oligodeoxynucleotide. Oligodeoxynucleotide (ODN) antisense and mismatch to PKCε were prepared as described previously (Parada et al., 2003; Dina et al., 2006). The antisense ODN, 5′-GCC AGC TCG ATC TTG CGC CC-3′, was directed against a unique sequence of rat PKCε. The corresponding GenBank (National Institutes of Health, Bethesda, MD) accession number and ODN position within the cDNA sequence are XM345631 and 226–245, respectively. We have previously shown that spinal intrathecal administration of antisense ODN with this sequence decreases PKCe protein in dorsal root ganglia (Parada et al., 2003). The sequence of the mismatch ODN, 5′-GCC AGC **G**CG ATC TT**T** CGC CC-3′, corresponds to the PKCε antisense sequence with 2 bases mismatched (in bold typeface). Control animals received injections of mismatch ODN.

Before use, lyophilized ODN was reconstituted in nuclease-free 0.9% NaCl to a concentration of 5 μg/μl and stored at −20°C until use. A dose of 20 μg of antisense or mismatch ODN was intrathecally administered in a volume of 20 μl once daily for 3 d. Before each injection, rats were anesthetized with 2.5% isoflurane containing oxygen. ODN was injected using a 30-gauge needle inserted between the fifth and sixth lumbar vertebrae, at the level of the cauda equina.

Protocol to induce opioid tolerance. PGE_2 induces dose-dependent mechanical hyperalgesia when injected intradermally in the dorsum of the rat's hindpaw (Khasar et al., 1994; Parada et al., 2003). A single dose of DAMGO (1 μg), a μ-opioid receptor agonist, attenuated PGE_2 (100 ng)-induced hyperalgesia when injected with PGE_2 (Aley et al., 1995; Aley and Levine, 1997). However, 3 hourly injections of DAMGO produced tolerance to the antinociceptive effect of a fourth hourly injection (Aley and Levine, 1997). This protocol of 3 hourly administrations of DAMGO was employed in all opioid tolerance experiments.

Protocol to detect opioid dependence. The selective μ-opioid receptor antagonist CTOP, which had no effect on the paw withdrawal threshold of normal rats, produced withdrawal hyperalgesia when administered on the fourth hour following 3 hourly injections of DAMGO (Aley and Levine, 1997). This protocol was used, throughout this study, to test for opioid dependence.

Statistical analysis. Group data are presented as mean ± SEM of $n = 6$ or more observations in each experimental group. Statistical significance was determined by ANOVA followed by Scheffe's *post hoc* test; $p < 0.05$ was considered statistically significant.

Results

Hyperalgesic priming induced by a μ-opioid agonist

As previously reported (Aley and Levine, 1997), the intradermal coinjection of DAMGO (1 μg) with PGE_2 (100 ng) in the hindpaw of naive rats inhibits the mechanical hyperalgesia induced by the PGE_2 (Fig. 1). However, when the coadministration of DAMGO and PGE_2 was performed 1 h after 3 hourly injections of DAMGO, the DAMGO was no longer able to attenuate PGE_2 hyperalgesia (Fig. 1). In addition to this opioid tolerance, the 3 injections of DAMGO induced hyperalgesic priming. Thus, while PGE_2 hyperalgesia is short-lived (<4 h) in naive control rats, it is markedly prolonged in rats pretreated with 3 hourly injections of DAMGO, persisting unattenuated for at least 4 h, even when DAMGO is coinjected with PGE_2.

In a previous study (Aley et al., 1995), control experiments in which saline was injected in the same 3× hourly protocol showed no significant effect of the DAMGO vehicle on nociceptive threshold. In the present study, baseline nociceptive threshold was not significantly different between any of the experimental groups.

Hyperalgesic priming induced by DAMGO persists, similar to the long-lived priming induced by carrageenan (Parada et al., 2005), with PGE_2 hyperalgesia still enhanced when tested 4–5 d after 3 hourly injections of DAMGO (Fig. 1). Of note, while hyperalgesic priming induced by carrageenan (or even by the PKCε activator ψεRACK) requires 3–5 d to develop (Aley et al., 2000), that induced by 3 hourly injections of DAMGO is already present by 1 h after the last dose of DAMGO.

We have previously shown that the development of inflammation-induced hyperalgesic priming is PKCε-dependent (Aley et al., 2000). Therefore, we next determined whether the development of DAMGO-induced hyperalgesic priming is also PKCε-dependent. Spinal intrathecal administration of ODN antisense to PKCε, was used in a protocol (20 μg in a volume of 20 μl daily, for 3 d) shown previously to decrease PKCε expression and function in primary afferent nociceptors and to prevent carrageenan-induced hyperalgesic priming (Parada et al., 2003;

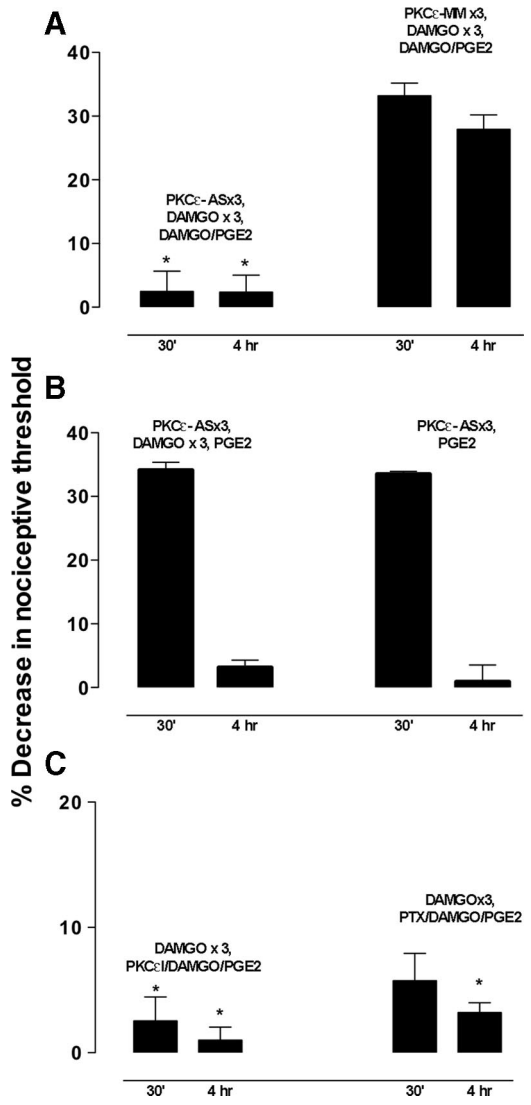

Figure 2. ***A***, Spinal intrathecal injection of oligodeoxynucleotide (ODN) antisense (PKCε-AS) but not mismatch (PKCε-MM) for PKCε (20 μg in a volume of 20 μl, i.t.), daily for 3 d, prevented the development of tolerance and priming when on the fourth day 3 hourly injections of DAMGOx3 was followed at the fourth hour by DAMGO plus PGE_2 (PKCε-ASx3, DAMGOx3, DAMGO/PGE_2, $^*p < 0.001$ compared with naive, $n = 6$ and PKCε-MMx3, DAMGOx3, DAMGO/PGE_2, $p =$ NS compared with naive, $n = 6$). ***B***, Spinal intrathecal injection of PKCε-AS for 3 d prevented priming (hyperalgesia at the fourth hour), when on the fourth day whether PGE_2 was injected with DAMGO, following 3 hourly injections of DAMGO (PKCε-ASx3, DAMGOx3, PGE_2, $p =$ NS, $n = 6$), or injected without DAMGO (PKCε-ASx3, PGE_2, $p =$ NS, $n = 6$); in both groups PGE_2 hyperalgesia at 30 min was not affected. ***C***, Intradermal injection of $PKC\varepsilon_{V1\text{-}2}$, a selective PKCε translocation inhibitor peptide (PKCεI, 1 μg), or pertussis toxin (PTX, 10 ng), a G_i-protein inhibitor, reversed (DAMGOx3-induced) tolerance and priming when injected 30 min (30′) before the fourth hourly injection of DAMGO plus PGE_2 (DAMGOx3, PKCεI/DAMGO/PGE_2, or DAMGOx3, PTX/DAMGO/PGE_2, both $^*p < 0.001$, $n = 6$/group).

Joseph et al., 2007). One day after the third and last injection of antisense or mismatch ODN, three hourly injections of DAMGO were administered, followed at the fourth hour by DAMGO plus PGE_2. The PKCε-antisense ODN pretreatment prevented the development of DAMGO-induced hyperalgesic priming (the hyperalgesia induced by PGE_2 was not enhanced or prolonged compared with that in mismatch ODN-treated rats) (Fig. 2*A*,*B*). PKCε-antisense ODN also restored the ability of DAMGO to inhibit PGE_2 hyperalgesia in rats treated with three hourly injections of DAMGO (DAMGOx3-treated rats) (Fig. 2*A*,*B*). To confirm that PGE_2-induced hyperalgesia was not attenuated by PKCε antisense, separate groups of rats were treated with antisense for 3 d and PGE_2 was administered on the fourth day following three hourly injections of DAMGO or without prior treatment with DAMGO. PGE_2 hyperalgesia was not attenuated by prior antisense treatment (Fig. 2*B*). The role of PKCε was confirmed using an alternative method of reducing PKCε activity, intradermal injection of the PKCε antagonist, $PKC\varepsilon_{V1\text{-}2}$ (1 μg) (Fig. 2*C*). In inflammation-induced hyperalgesic priming, there is a switch in the G-protein specie that mediates PGE_2-induced hyperalgesia, from G_s to G_i, as indicated by the development of sensitivity to inhibition by pertussis toxin (Dina et al., 2009). We found that pertussis toxin (10 ng) also attenuates PGE_2-induced hyperalgesia in DAMGO-primed rats (Fig. 2*C*).

μ-Opioid tolerance induced by inflammation

Having determined that the induction of μ-opioid tolerance also produces hyperalgesic priming, we next tested whether, conversely, μ-opioid tolerance is produced when intradermal injection of carrageenan or a direct activator of PKCε, ψεRACK, induces hyperalgesic priming. Injection of carrageenan (5 μl of a 1% solution) or ψεRACK (1 μg) produced hyperalgesia that lasted ~3 d (data not shown). On day 5 following injection of carrageenan or ψεRACK, when nociceptive threshold had returned to pretreatment baseline, DAMGO did not inhibit PGE_2 hyperalgesia (Fig. 3*A*). However, administration of ODN antisense, but not mismatch, to PKCε, for 3 d before injection of carrageenan or ψεRACK, blocked the development of tolerance for inhibition of PGE_2 hyperalgesia by DAMGO, even when measured 5 d after administration of carrageenan or ψεRACK (Fig. 3*B*). Tolerance to the analgesic effect of the potent μ-opioid agonist DAMGO, induced by intradermal injection of carrageenan and ψεRACK, was also attenuated by intradermal injection of the PKCε antagonist, $PKC\varepsilon_{V1\text{-}2}$ (Fig. 3*C*), and the G_i-protein inhibitor, pertussis toxin (Fig. 3*D*), demonstrating a role for PKCε and G_i in the peripheral terminals of the primary afferent nociceptor.

μ-Opioid dependence induced by inflammation: PKCε and G_i mediation of opioid withdrawal hyperalgesia

We have previously shown that the protocol of 3 hourly intradermal injections of DAMGO, also induces μ-opioid receptor dependence, demonstrated by the ability of the μ-opioid receptor antagonist, CTOP (1 μg), to induce mechanical hyperalgesia (Aley et al., 1995). In the present study we found that this CTOP-induced hyperalgesia (Fig. 4*A*) was inhibited by the PKCε antagonist, $PKC\varepsilon_{V1\text{-}2}$, and by the G_I inhibitor, pertussis toxin (Fig. 4*A*). Furthermore, we found that hyperalgesic priming induced by either carrageenan or ψεRACK (which induce μ-opioid tolerance) also induced μ-opioid dependence (Fig. 4*B*,*C*). CTOP was injected 5 d after carrageenan or ψεRACK administration, when nociceptive threshold had returned to baseline. In both groups of rats (neither of which had been exposed to μ-opioid agonist) CTOP induced mechanical hyperalgesia (Fig. 4*B*,*C*). The PKCε antagonist $PKC\varepsilon_{V1\text{-}2}$ and the G_i-protein inhibitor pertussis toxin also attenuated this hyperalgesia (Fig. 4*B*,*C*).

Discussion

We demonstrate that repeated administration of a μ-opioid agonist, DAMGO to produce tolerance and dependence to the peripheral analgesic action of the opioid produces hyperalgesic priming, and conversely, that induction of hyperalgesic priming by inflammation produces opioid tolerance and dependence. Furthermore, both effects are produced via PKCε-dependent

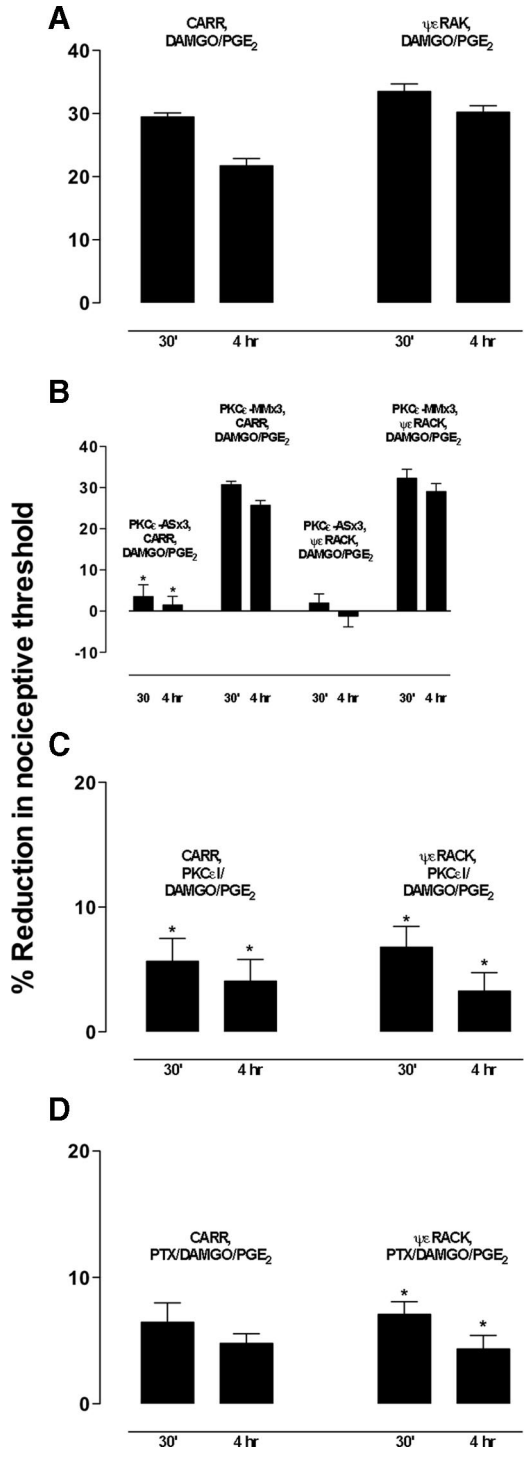

Figure 3. ***A***, DAMGO did not attenuate PGE_2-induced hyperalgesia in carrageenan (CARR, 5 μl of 1% solution)- or protein kinase Cε activator ($\psi\varepsilon$RACK, 1 μg)-pretreated (5 d prior) rats (CARR, fifth day DAMGO/PGE_2, p = NS, $n = 6$; $\psi\varepsilon$RACK, fifth day DAMGO/PGE_2, p = NS, $n = 6$). PGE_2 hyperalgesia was still present at the fourth hour in the CARR- and $\psi\varepsilon$RACK-pretreated rats. ***B***, Spinal intrathecal injection of antisense (PKCε-AS) but not mismatch (PKCε-MM) ODN for PKCε (20 μg in a volume of 20 μl), for three d, prevented the development of tolerance and priming when CARR or $\psi\varepsilon$RACK were injected on the fourth day and the effect of DAMGO on PGE_2 hyperalgesia was tested 5 d after the administration of CARR or $\psi\varepsilon$RACK. (PKCε-ASx3, CARR, DAMGO/PGE_2 and PKCε-ASx3, $\psi\varepsilon$RACK, DAMGO/PGE_2, both $^*p < 0.001$, $n = 6$/group; PKCε-MMx3, CARR, DAMGO/PGE_2 and PKCε-MMx3, $\psi\varepsilon$RACK, DAMGO/PGE_2, both p = NS, $n = 6$/group). ***C***, Intradermal injection of $PKC_{\varepsilon V1\text{-}2}$, a selective PKCε translocation inhibitor peptide (PKCεI, 1 μg), also reversed tolerance and priming when PKCεI was injected 30 min (30′) before DAMGO/PGE_2 on the fifth day following the administration of CARR or $\psi\varepsilon$RACK (CARR, PKCεI/DAMGO/PGE_2 and $\psi\varepsilon$RACK, PKCεI/DAMGO/PGE_2, both $^*p < 0.001$, $n = 6$/group). ***D***, Intradermal injection of pertussis toxin, a G_i inhibitor (PTX, 10 ng), also reversed tolerance and priming when it was injected 30 min (30′) before DAMGO/PGE_2 on the fifth day following the administration of CARR or $\psi\varepsilon$RACK (CARR, PTX/DAMGO/PGE_2 and $\psi\varepsilon$RACK, PTX/DAMGO/PGE_2, both $^*p < 0.001$, $n = 6$/group).

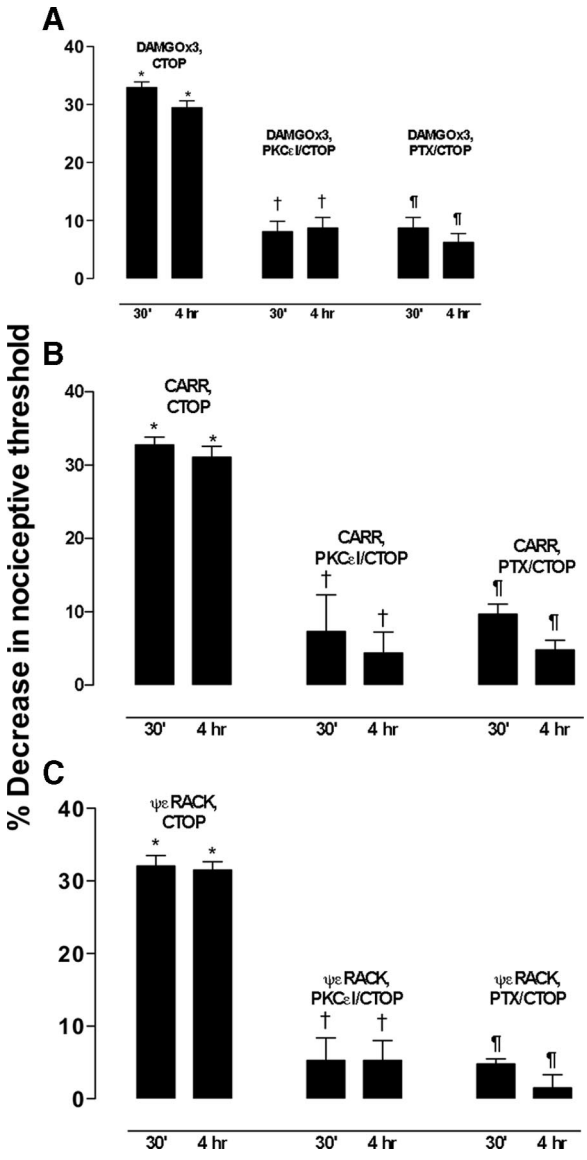

Figure 4. ***A***, Intradermal injection of CTOP (1 μg), a selective μ-opioid antagonist, at the fourth hour following three hourly injections of DAMGO produced hyperalgesia (DAMGOx3, CTOP, $^*p < 0.001$ compared with baseline, $n = 6$) and this hyperalgesia was still present at the fourth hour after CTOP administration. Injection of PKCε inhibitor ($PKC_{\varepsilon V1\text{-}2}$) or the G_i-protein inhibitor PTX 30 min (30′) before CTOP reversed CTOP hyperalgesia (DAMGOx3, PKCεI/CTOP, $^{\dagger}p < 0.001$, $n = 6$; DAMGOx3, PTX/CTOP, $^{\P}p < 0.001$, $n = 6$). ***B***, Intradermal injection of CTOP in CARR-pretreated rats (5 d prior) produced significant hyperalgesia (CARR, fifth day, CTOP, $^*p < 0.001$, $n = 6$). Injection of $PKC_{\varepsilon V1\text{-}2}$ or PTX 30 min (30′) before CTOP reversed CTOP hyperalgesia (CARR, PKCεI/CTOP, $^{\dagger}p < 0.001$, $n = 6$; CARR, PTX/CTOP, $^{\P}p < 0.001$, $n = 6$). ***C***, Intradermal injection of CTOP in $\psi\varepsilon$RACK-pretreated rats (5 d prior) produced significant hyperalgesia ($\psi\varepsilon$RACK, fifth day, CTOP, $^*p < 0.001$, $n = 6$). Injection of $PKC_{\varepsilon V1\text{-}2}$ or the PTX 30 min (30′) before CTOP reversed CTOP hyperalgesia ($\psi\varepsilon$RACK, PKCεI/CTOP, $^{\dagger}p < 0.001$, $n = 6$; $\psi\varepsilon$RACK, PTX/CTOP, $^{\P}p < 0.001$, $n = 6$).

mechanisms in primary afferents. These findings suggest that both the transition from persistent acute pain to a chronic pain state and the loss of responsiveness to opioid analgesics result from a single PKCε-dependent neuroplastic change in the primary

afferent nociceptor. This idea is compatible with the common clinical observation that a critical transition occurs in patients when opioid-sensitive "persistent acute pain" transforms into an opioid-resistant "chronic pain" state. Reversal of neuroplastic changes associated with hyperalgesic priming might provide a new therapeutic strategy for reinstating sensitivity to opioid analgesics in patients suffering from intractable chronic pain.

The mechanistic interactions between the transition to chronic pain and the development of resistance to opioid analgesics at the level of the peripheral terminal of the primary afferent nociceptor may play a role in other clinically observed interactions between opioids and chronic pain that are otherwise difficult to explain (Fig. 5). For example, chronic use of opioid analgesics can contribute to the transition from acute intermittent pain to chronic pain in patients with migraine and other types of headache (Mathew et al., 1982; Wilkinson et al., 2001; Biondi, 2003; Bigal and Lipton, 2009) and may also contribute to the "chronification" of low back (Webster et al., 2007; Franklin et al., 2008), and other pain conditions (Compton, 1994; Mao et al., 1995; Savage, 1996). Chronic opioid use can also produce or enhance ongoing pain, a phenomenon referred to as "opioid-induced hyperalgesia" (Mercadante and Arcuri, 2005; Chu et al., 2008; Chen et al., 2009; Hay et al., 2009). Although opioid-induced hyperalgesia was not observed in the present study, we hypothesize that opioid-induced hyperalgesia may originate in part from PKCε-dependent mechanisms (Chu et al., 2008) related to those that mediate hyperalgesic priming and opioid tolerance/dependence.

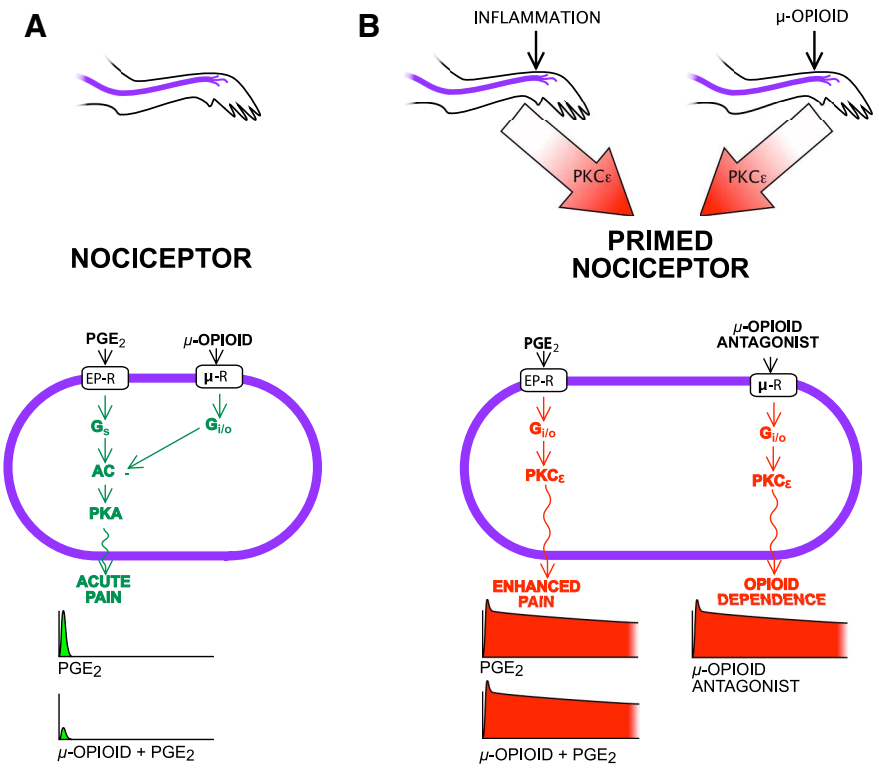

Figure 5. A proposed mechanism relating the transition to chronic pain and the loss of analgesic efficacy. ***A***, In a nociceptor (purple) in the hindpaw of a naive animal, a proinflammatory cytokine (PGE_2), causes acute pain (hyperalgesia lasting <4 h) that is mediated by activation of a G_s-protein-coupled receptor for prostaglandin (EP-R) causing increased adenylyl cyclase (AC) activity and activation of PKA, ultimately leading to increased membrane excitability and nerve activity, which underlies acute pain. In the naive animal, μ-opioids act at their receptor (μ-R) to inhibit the second messenger pathway mediating acute pain (μ-R activates a $G_{i/o}$ protein which inhibits AC). ***B***, The transition to chronic pain and loss of analgesic efficacy is due to a change in second messenger signaling in the nociceptor (priming). This change can be induced by exposure either to a proinflammatory cytokine or to an opioid analgesic. In this primed state, both the prostaglandin and opioid receptors become coupled to PKCε (via $G_{i/o}$) and can produce prolonged hyperalgesia, a model of chronic pain and opioid tolerance and dependence.

We found that the μ-receptor antagonist CTOP reduced the hyperalgesic priming that followed a series of injections of the μ-receptor agonist DAMGO. One potential explanation for this observation is that CTOP antagonized an action of residual DAMGO remaining at the injection site. This seems unlikely, however, in view of the very small amount of DAMGO injected (1 μg) and because the enhancement of nociception is not observed when a μ receptor antagonist is injected with DAMGO (upon first injection of DAMGO in an opioid-naive animal) (Aley et al., 1995). Of note in this regard, the literature on opioid tolerance describes a mechanism by which opiate analgesics can produce a change in the response to CTOP that outlasts the presence of opiate. Thus, exposure to μ-agonists (including DAMGO) can transform the μ-opioid receptor into an agonist-independent constitutively active state (Liu and Prather, 2001), revealing an inverse agonist action of CTOP (Brillet et al., 2003). We speculate that such constitutive μ-receptor activity occurs in hyperalgesic priming, and accounts for our observations with CTOP. The constitutive activity might be induced either by the exogenous DAMGO or, when carrageenan is the priming agent, by inflammogen stimulated release of endogenous opioids during the 3 d of carrageenan-induced hyperalgesia (Wang et al., 2004).

The known cellular mechanisms of hyperalgesic priming are similar (PKCε dependence and G-protein switch in cytokine hyperalgesia), whether the priming is induced by an inflammogen, a direct PKCε activator, a μ-opioid agonist, or stress. However, priming induced by the μ-opioid DAMGO differs in one notable respect; opioid-induced priming develops in <4 h, while that induced by inflammation requires 3–5 d (Aley et al., 2000) and that by sound stress, 1–2 weeks (Khasar et al., 2008). Such rapid onset of opioid-induced hyperalgesic priming may provide important insights for future investigation of the PKCε-dependent pathways that mediate hyperalgesic priming. Thus, mechanisms such as transcription and translation, which would seem compatible with the longer time course of hyperalgesic priming induced by inflammation or stress seem much less feasible in the under-four-hour timeframe of opioid-induced priming. This suggests the possibility that opioids induce priming by engaging a cellular mechanism downstream to that engaged by the other inducers of hyperalgesic priming that we have investigated.

Our finding of opioid-induced hyperalgesic priming also provides some insight into mechanisms underlying the prolonged hyperalgesia induced by PGE_2. While we have previously shown that the hyperalgesia induced by PGE_2, following induction of hyperalgesic priming remains protein kinase A dependent (Aley et al., 2000), the present finding that pertussis toxin inhibits PGE_2 hyperalgesia at the 30 min time point indicates that in the primed state this PKA-dependent hyperalgesia is G_i, not G_s, dependent.

We have recently demonstrated that chronic unpredictable stress also produces hyperalgesic priming in the primary afferent (Khasar et al., 2008; Dina et al., 2009). Because stress-induced hyperalgesic priming exhibited PKCε dependence similar to the PKCε dependence that we now know is shared by inflammation-induced and opioid-induced priming, we predict that interactions among stress, inflammation, and opioids at the level of primary afferent intracellular signaling pathways may contribute to the generation of opioid-resistant chronic pain states. Consistent with this idea, stress-induced analgesia can be cross-tolerant with morphine-induced analgesia (Lewis et al., 1981; Girardot and Holloway, 1984; Szikszay and Benedek, 1989; da Silva Torres et al., 2003; Fazli-Tabaei et al., 2005). Thus, interaction among stress, opioids and hyperalgesic priming at the level of the primary afferent nerve ending may be important in pain patients in which all three factors often coexist.

It is likely that the cell signaling interactions between different inducers of hyperalgesic priming are not limited only to PKCε and G-protein switching. For example, phospholipase Cβ3 (PLCβ3) also contributes to inflammatory mediator-induced mechanical hyperalgesia and μ-opioid analgesia. Specifically, we and others have demonstrated the presence of PLCβ3 in small-diameter dorsal root ganglion neurons (Han et al., 2006; Joseph et al., 2007; Shi et al., 2008), and provided evidence that it is upstream of PKCε in nociceptor sensitization and hyperalgesic priming (Joseph et al., 2007). μ-opioid agonists have also been shown to activate PLC (Ono et al., 2002; Galeotti et al., 2006; Mathews et al., 2008), by releasing β/γ subunits from $Gi\alpha_{2/o}$ (Murthy and Makhlouf, 1996; Xie et al., 1999; Bianchi et al., 2009), which in turn might contribute to the paradoxical hyperalgesia induced by chronic opioid administration (Rosenblum et al., 2008). PLCβ3 also contributes to μ-opioid tolerance and dependence (Mestek et al., 1995; Smith et al., 1999; Rosenblum et al., 2008). While PLCβ3 has been shown to be a downstream target of the cAMP-activated guanine exchange factor, Epac (Hucho et al., 2005), the exact relationship of the PLCβ3 contribution to those of PKCε and G_i-proteins, in the transition to chronic pain and μ-opioid receptor tolerance and dependence remains to be established.

Our findings in the peripheral nervous system may also have relevance to interactions between chronic pain and opioid analgesics in the CNS. Thus, μ-opioid receptors are also located on the central terminals of primary afferents in the spinal cord and trigeminal dorsal horn (Kline and Wiley, 2008) where they contribute to the analgesic effect of systemically administered opioids (Aicher et al., 2000; Kohno et al., 2005). There is abundant evidence for PKC signaling and G-protein switching in opioid tolerance and dependence at spinal and supraspinal sites (Mestek et al., 1995; Liu and Anand, 2001; Sánchez-Blázquezet al., 2001; Chakrabarti et al., 2005; Wang and Burns, 2006; Kelly et al., 2008). Similarly, in PKCε knock-out mice, systemic opioids induce both enhanced analgesia and decreased opioid tolerance (Newton et al., 2007). Furthermore, morphine has been shown to induce rapid and marked desensitization of μ-opioid receptors in locus ceruleus neurons, but only when protein kinase C is activated (Bailey et al., 2004).

Any potential role that hyperalgesic priming might play in the CNS would be in addition to other well documented central mechanisms likely to play a role in the opioid-resistance that characterizes chronic pain states. For example, opioid activation of astrocytes and microglia in the CNS may play an important role in neuropathic pain and opioid tolerance (Watkins et al., 2009). (In contrast, it seems unlikely that a similar effect of opioids on the sparse glial cells in the skin could play an important role in the peripheral effects we have described.) Another important mechanism of interactions between opiate use and chronic pain in the CNS is pain-related increases in expression of cholecystokinin (the "anti-opioid") that can antagonize opiate analgesia (Wiesenfeld-Hallin et al., 2002).

In conclusion, the present experiments demonstrate shared mechanisms between a transition from acute to chronic pain and the development of μ-opioid tolerance and dependence. These observations provide insight into possible cellular mechanisms of the opioid-resistance that characterizes many chronic pain states, as well as clues toward possible avenues in our search for novel approaches to address the great suffering and societal expense caused by intractable chronic pain.

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