Intrathecal Etanercept Partially Restores Morphine's Antinociception in Morphine-Tolerant Rats via Attenuation of the Glutamatergic Transmission

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BACKGROUND: Long-term exposure to morphine leads to analgesic tolerance. In addition to an opioid receptor conformational change, enhancing the glutamatergic signal transmission is also involved in morphine tolerance. Tumor necrosis factor-α has been demonstrated to correlate with neuronal plasticity via activation of glutamatergic transmission. We examined the effect of etanercept, a tumor necrosis factor-α inhibitor on morphine tolerance in rats.

METHODS: Male Wistar rats were implanted with 2 intrathecal (IT) catheters, and 1 IT catheter was connected to a mini-osmotic pump, used for either morphine infusion (15 μg/h) or saline (1 μL/h) infusion for 5 days. On day 5, either etanercept (50 μg) or saline (10 μL) was injected after discontinued morphine infusion. Three hours later, acute morphine (15 μg/10 μL, IT) treatment was given and all rats received a nociceptive tail-flick test.

RESULTS: The results showed that acute etanercept (50 μg) treatment caused a significant antinociceptive effect of morphine in morphine-tolerant rats. Western blotting indicated that etanercept attenuated the downregulation of membrane glutamate transporters GLT-1 and GLAST in morphine-tolerant rats. Etanercept also inhibited the upregulation of surface AMPA-receptor and *N*-methyl-D-aspartate–receptor subunits, including GluR1/GluR2 and NR1/NR2A.

CONCLUSIONS: These results demonstrate that etanercept partially restores the antinociceptive effect of morphine in morphine tolerance after a morphine challenge. Etanercept has potential for use in the clinical management of pain, particularly in patients who require long-term opioid treatment, and the effectiveness of which can be hampered by tolerance. (Anesth Analg 2011; X:000–000)

Opioids are potent analgesics and widely used for pain management. Long-term use of opioids leads to tolerance, which limits their utility. Morphine tolerance is a pharmacological phenomenon characterized by a shift of the dose-response curve to the right with a larger 50% effective dose.[1] Koob and Bloom[2] described 2 possible mechanisms of drug tolerance: within-system and between-system adaptation. Investigations of the mechanisms of within-system opioid adaptation include examination of the role of intracellular adenylyl cyclase and cyclic adenosine monophosphate–dependent protein kinase A,[3–5] G protein uncoupling,[6,7] β-arrestin binding and internalization of opioid receptors,[8–10] and μ-opioid receptor oligomerization.[11,12] Studies of between-system adaptation have focused on glutamatergic synaptic transmission via activation of *N*-methyl-D-aspartate (NMDA) receptor function,[13–17] downregulation of glutamate transporters (GTs),[17,18] glia activation with increasing proinflammatory cytokine expression,[19,20] and apoptosis.[18]

Tumor necrosis factor (TNF)-α, a 17-kDa peptide, is constitutively expressed in neurons and glial cells of the central nervous system[21] and is released from neurons and glia upon stimulation.[22–24] In H4 astroglioma cells, TNF-α and corepressor N-myc suppress EAAT2/GLT-1 gene expression.[25] TNF-α may also enhance synaptic strength by increasing the expression of the AMPA receptor.[26] Hence, TNF-α could regulate the expression of GTs and receptors and influence the function of the glutamatergic system. Previous reports suggest that TNF-α has an important role in the function of the neural circuit by modulating glutamatergic synaptic transmission.[27,28]

TNF inhibitors have been widely used for the treatment of rheumatoid arthritis (RA) and other inflammatory autoimmune diseases.[29] Etanercept (Enbrel; Amgen, Thousand Oaks, CA, and Wyeth) is a recombinant soluble p75 receptor that binds to the released TNF-α and prevents its interaction with membrane receptors, thereby modulating TNF-α–mediated signal transduction.[30] Etanercept has potential for treatment of inflammatory conditions characterized by an excess of TNF-α, such as ankylosing spondylitis,[31] Crohn disease,[32] and Alzheimer disease.[33] Etanercept is approved for the treatment of RA, juvenile RA, psoriasis, ankylosing spondylitis, and psoriatic arthritis in the United States. In the present study, we examined the effect of a

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single intrathecal (IT) etanercept injection on the antinociceptive effect of morphine in morphine-tolerant rats and its underlying mechanisms.

METHODS

Animal Preparation and IT Drug Delivery

Eight-week-old male Wistar rats (300–350 g, $n = 112$) were sedated with pentobarbital (50 mg/kg, intraperitoneally; Sigma, St. Louis, MO) and then under isoflurane anesthesia. All rats were implanted via the atlantooccipital membrane with 2 IT polyethylene (PE)-10 catheters (0.8 cm), which were advanced caudally to the lumbar enlargement of the spinal cord. The level of L1-2 spinal bony structure corresponds to the spinal cord segments L5, L6, and S1-3, which are responsible for the tail flick reflex.[34] The ends of the catheters were externalized and fixed to the dorsal aspect of the head. One IT catheter was connected to a mini-osmotic pump, implanted in the interscapular region, and used for either morphine (15 μg/h) (Sigma) or saline (1 μL/h) infusion for 5 days. After the operation, all rats were returned to their home cages for recovery; each rat was housed individually and maintained on a 12-hour light/dark cycle with food and water freely available. During the experiment, any rats that developed any abnormal behavior (such as poor appetite, decreased stool passage, decreased daily activity, frequent vocalizations, or flaccidity), motor deficit, or obstructed IT catheter were excluded from the experiments. The use of rats conformed to the Guiding Principles in the Care and Use of Animals of the American Physiology Society and was approved by the National Defense Medical Center Animal Care and Use Committee.

Construction of the IT Catheter

The IT catheter was constructed using an 8-cm PE tube (0.008-in. inner diameter, 0.014-in. outer diameter; Spectranetics, Colorado Springs, CO) and a 3.5-cm silastic tube (Dow Corning, Midland, MI). The silastic tube was inserted into the PE tube and the joint sealed with epoxy resin and silicon rubber. The dead space of the IT catheter was approximately 8 μL.

Nociceptive Test

The antinociception test was modified and adapted from our previous studies.[19,35] Tail-flick latency, by the hot water immersion test (52°C ± 0.5°C), was measured before drug infusion and daily after the start of drug infusion for 5 days. The baseline latency was approximately 1.95 ± 0.04 seconds with a cutoff time of 10 seconds. Rats were placed in plastic restrainers for drug injection and antinociception assessment. All drug infusions were performed at the rate of 1 μL/h via a mini-osmotic pump (Alzet, Cupertino, CA). On day 5, the IT catheter for drug infusion was cut, and either etanercept (50 μg in 10 μL; Enbrel, Amgen and Wyeth) or saline (10 μL) was administered via the other IT catheter. Three hours later, a morphine challenge test (15 μg/10 μL saline) was performed and antinociceptive response was examined for each study group. The tail-flick latency was recorded at 30, 60, 90, and 120 minutes after morphine administration. The areas under the curve (AUCs) were from time 0 to 120 minutes for measurement of the antinociceptive effect of morphine.

Spinal Cord Preparation

Three hours after discontinuation of the IT drug infusion and simultaneous infusion of etanercept or saline treatment on day 5, rats were killed by exsanguinations under isoflurane anesthesia (ABBOTT; Abbott Laboratories, Ltd., Queenborough, Kent, UK). Laminectomy was then performed at the lower edge of the 12th thoracic vertebra; the L5-S1 segment of the spinal cord was removed immediately and separated into ventral and dorsal parts. The dorsal parts were stored at −80°C until used for Western blotting.

Preparation of Spinal Cord Plasma Membrane, Cytosolic, and Nuclear Fractions for Western Blotting Analysis

Each dorsal section of the spinal cords was fractionated into cytosolic, membrane, and nuclear fractions with the cytoplasmic, nuclear, and membrane compartment protein extraction kit as recommended by the manufacturer (BioChain Institute, Inc., Hayward, CA). The membrane fraction was checked by Western blotting with anti-EGFR (epidermal growth factor receptor) (MBL, Naka-ku, Nagoya, Japan) for specificity.

Western Blotting Analysis

The membrane fractions were denatured by heating at 95°C for 10 minutes in an equal volume of sodium dodecyl sulfate (SDS) sample buffer, then separated on SDS–10% polyacrylamide gel electrophoresis, and transferred to a polyvinylidene difluoride membrane (Immobilon™; Millipore, Bedford, MA). The membrane was blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 and incubated overnight at 4°C with polyclonal guinea pig antirat GLT-1 (1:5000) or antirat GLAST antibodies (1:5000), polyclonal rabbit antirat EAAC1 antibodies (1:5000) or rabbit antirat GluR1 (1:5000), or GluR2 (1:5000), or NR1 (1:5000) (all from Chemicon, Temecula, CA), then with the corresponding horseradish peroxidase–conjugated secondary antibody (1:5000, donkey antiguinea pig or goat antirabbit immunoglobulin G or goat antimouse immunoglobulin G, all from Chemicon) for 1 hour at room temperature. After reaction with ECL solution (Amersham, Arlington Heights, IL), the bound antibody was visualized using a chemiluminescence imaging system (Syngene, Cambridge, UK). Finally, the blots were incubated at 56°C for 18 minutes in stripping buffer (0.0626 mol/L Tris-HCl, pH 6.7, 2% SDS, 0.1 mol/L mercaptoethanol) and reprobed with a monoclonal mouse anti-EGFR (Sigma) as the loading control. The optical density of each specific band was measured using a computer-assisted imaging analysis system (GeneTools Match software; Syngene).

Data and Statistical Analysis

All data are presented as the mean ± SEM where n is the number of rats used. Tail-flick latency data were analyzed using 2-way analysis of variance with post hoc Bonferroni test. A significant difference was defined as a P value <0.05. For immunoreactivity data, the intensity of each test band was expressed as the relative optical density

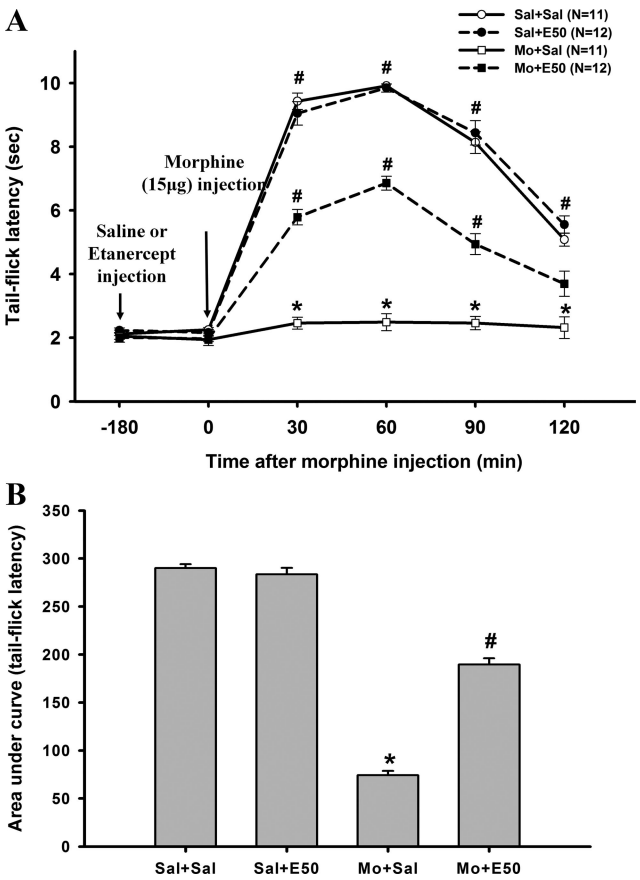

Figure 1. Etanercept partially restores the antinociceptive effect of morphine in morphine-tolerant rats. A, Effect of etanercept on the antinociceptive effect of morphine challenge was examined on day 5. Saline or etanercept (50 μg in 10 μL) was given intrathecally after discontinuation of saline or morphine infusion, and morphine challenge (15 μg in 10 μL) was performed 3 hours later. Tail-flick latency was measured every 30 minutes for 120 minutes. The 0-minute time point was 3 hours after etanercept was given. B, The area under the curve from time 0 to 120 minutes is shown. #$P < 0.05$ compared with the control group (Sal + Sal); *$P < 0.05$ compared with the morphine-tolerant group (Mo + Sal). All data are expressed as mean ± SEM and averaged from the indicated number of rats. Sal + Sal = saline infusion + saline treatment; Sal + E50 = saline infusion + etanercept (50-μg) treatment; Mo + Sal = morphine infusion + saline treatment; Mo + E50 = morphine infusion + etanercept (50-μg) treatment.

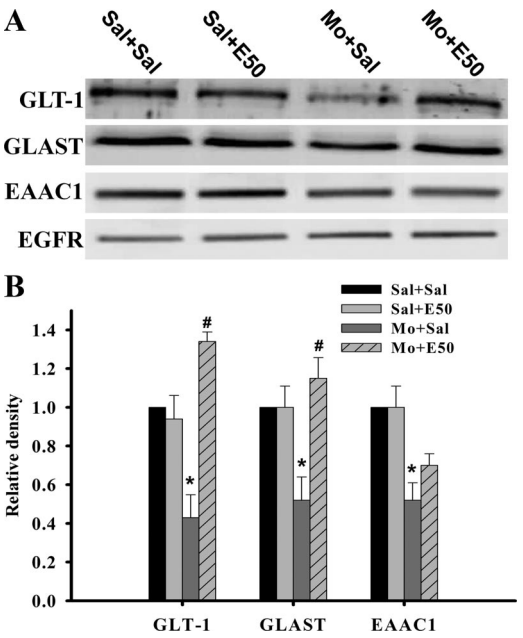

Figure 2. Etanercept prevents the downregulation of the membrane-bound glutamate transporters in the dorsal horn of morphine-tolerant rat spinal cords. A, Western blot analysis shows levels of GLAST/GLT-1/EACC1 in the dorsal horn of spinal cords from 4 treatment groups. B, Display of the laser densitometric analysis of protein bands after normalization with β-actin. Data are mean ± SEM and averaged from 6 rats of each group. #$P < 0.05$ compared with the control group; *$P < 0.05$ compared with the morphine-tolerant group. Sal + Sal = saline infusion + saline treatment; Mo + Sal = morphine infusion + saline treatment; Sal + E50 = saline infusion + etanercept (50-μg) treatment; Mo + E50 = morphine infusion + etanercept (50-μg) treatment.

calculated with respect to the average optical density for the corresponding control band. For statistical analysis, immunoreactivity was analyzed using 1-way analysis of variance, followed by multiple comparisons with the Bonferroni test. A significant difference was defined as a P value <0.05.

RESULTS

Etanercept Restored the Antinociceptive Effect of Morphine in Chronic Morphine-Infused Rats

Similar to our previous studies,[17,19] morphine challenge (15 μg, IT) produced an antinociceptive effect in saline-infused rats (AUC = 934.5 ± 13.12 minutes × seconds), but not in morphine-infused rats (AUC = 286.43 ± 9.40 minutes × seconds) 3 hours after discontinuation of pump infusion on day 5. Etanercept (50 μg) did not alter the tail-flick latencies in saline-infused rats and morphine-infused rats before morphine challenge, as shown in Figure 1A. However, a significant antinociceptive effect of the morphine challenge was observed in rats that received etanercept (50 μg) and showed an AUC = 622.61 ± 22.52 minutes × seconds, as shown in Figure 1B.

Etanercept Treatment Inhibited the Downregulation of Membrane GTs After Morphine Challenge in Chronic Morphine-Infused Rat Spinal Cord

We found a 50% to 60% reduction on the expression of membrane GTs GLAST, GLT-1, and EAAC1 in the dorsal horn of morphine-tolerant rat spinal cords (Fig. 2). This phenomenon was not observed in rats that received IT saline infusion followed by either saline or etanercept treatment. Etanercept (50 μg) not only restored the level of GLAST and GLT-1 expression in the dorsal horns of morphine-tolerant rat spinal cords, but exceeded the levels in the control group. However, the expression of EAAC1 was unaffected by etanercept (50 μg) in morphine-tolerant rats.

Chronic Morphine Infusion Upregulates Surface AMPA- and NMDA-Receptor Subunit Expression in Rat Spinal Cords

We also examined the expression of ionotropic glutamate receptors on the plasma membrane in the dorsal horn of

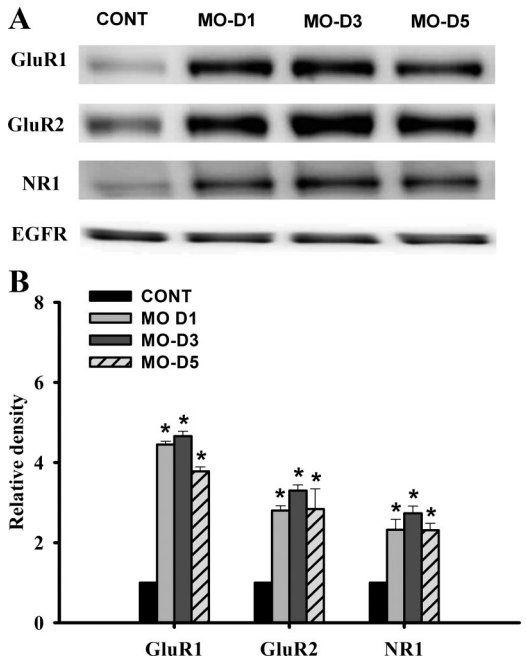

Figure 3. Time course of expression of AMPA-receptor and *N*-methyl-D-aspartate–receptor subunits in the dorsal horn of morphine-tolerant rat spinal cords. Rats were killed at day 1, 3, or 5 after intrathecal morphine infusion (15 μg/h). The L5-S3 segment of spinal cord was removed and dorsal parts were used for Western blot analysis. A, Western blot analysis of GluR1, GluR2, and NR1 subunits in the dorsal horn of spinal cords and spinal cord samples were harvested at days 1, 3, and 5 after long-term intrathecal morphine infusion (15 μg/h). B, Quantification of the data from A. EGFR (epidermal growth factor receptor) was used as the loading control. Data are averaged from 6 rats per group. #$P < 0.05$ compared with the control group. *$P < 0.05$ compared with the morphine-tolerant group.

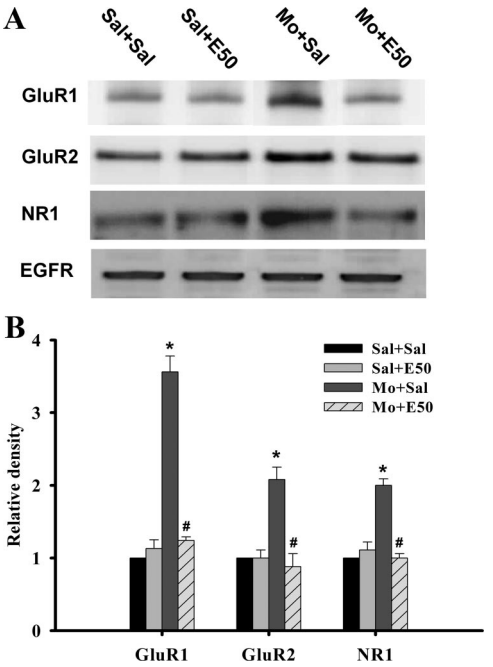

Figure 4. Effect of etanercept on expression of subunits of AMPA and *N*-methyl-D-aspartate receptors in morphine-tolerant rats. A, Western blot of GluR1, GluR2, and NR1 subunits in the dorsal horn of the spinal cords was performed after either morphine (15 μg/h) or saline (1 μL/h) infusion for 5 days and etanercept (50 μg) or saline treatment. B, Display of the laser densitometric analysis of protein bands after normalization with EGFR (epidermal growth factor receptor). Data are mean ± SEM and averaged from 6 rats of each group. #$P < 0.05$ compared with the control group; *$P < 0.05$ compared with the morphine-tolerant group. Sal + Sal = saline infusion + saline treatment; Mo + Sal = morphine infusion + saline treatment; Sal + E50 = saline infusion + etanercept (50-μg) treatment; Mo + E50 = morphine infusion + etanercept (50-μg) treatment.

morphine-tolerant rat spinal cords. The time-course changes of AMPA and NMDA receptors in chronic morphine-infused rats showed that there was an increase in the expression of GluR1, GluR2, and NR1 subunits on days 1, 3, and 5 (Fig. 3, A and B; $P < 0.05$).

Etanercept Attenuated AMPA and NMDA Receptor Expression on the Plasma Membrane of Chronic Morphine-Infused Rat Spinal Cord

On day 5, the expression of GluR1 was increased by 3.83-fold, GluR2 increased by 2.27-fold, and NR1 increased by 2.17-fold after morphine infusion, compared with the control group (Fig. 4, A and B). The enhancement on the expression of AMPA- and NMDA-receptor subunits on the plasma membrane was completely abolished by etanercept (50 μg) treatment in morphine-tolerant rats (Fig. 4, A and B; $P < 0.05$).

DISCUSSION

In this study, we demonstrated that the TNF-α inhibitor etanercept partially restores the antinociceptive effect of morphine in morphine-tolerant rats. Etanercept prevented not only the downregulation of the glial membrane GTs GLT-1 and GLAST, but also attenuated the upregulation of surface AMPA and NMDA receptors. Etanercept has been used to treat RA since 1998[29] and has been widely studied in the treatment of inflammatory disorders. A pilot study indicated that perispinal administration of etanercept dramatically improves cognitive function of Alzheimer patients.[33] In our present study, IT etanercept (50 μg) pretreatment restored the antinociceptive effect of morphine in morphine-tolerant rats.

Emerging evidence suggests that opioid tolerance and abnormal pain sensitivity may share common cellular mechanisms and be mediated, at least in part, through the glutamatergic receptor system.[36] The glutamatergic receptor system, especially NMDA receptors, is critically involved in long-term opioid-induced neuronal adaptations, such as opioid tolerance, dependence, and withdrawal.[13,17,37] Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system. Excess glutamate in the synaptic cleft leads to NMDA receptor activation and Ca^{2+} influx, which causes neuronal plasticity and even excitotoxicity[38]; glutamate homeostasis is crucial in maintaining efficient synaptic communication and prevention of neurotoxicity.[39] Glutamate is rapidly cleared from the synaptic cleft by diffusion and reuptake in normal intact brain tissue without compromised energy supply.[40] The reuptake is accomplished by GTs localized on the cytoplasmic membrane of astrocytes and neurons.[40,41] Mao et al.[18] demonstrated a correlation among GT downregulation, morphine tolerance, and thermal hyperalgesia in chronic

morphine-treated but not saline-treated rats. Similarly, our previous studies also demonstrated that coinfusion of the NMDA antagonist MK801, dexamethasone, and amitriptyline prevented GT downregulation, and the subsequent EAA elevation in the rat spinal CSF dialysates after morphine challenge, thus retained the antinociceptive effect of morphine.[17,19,42] In the present study, we examined the membrane GT expression in morphine-tolerant rats instead of total lysate because only GTs on the plasma membrane reuptake glutamate from the synaptic cleft. In addition, our previous study demonstrated a rapid GT trafficking from cytoplasm onto the plasma membrane by amitriptyline pretreatment before the morphine challenge in morphine-tolerant rats.[35] We therefore examined the membranous GTs and found that chronic morphine infusion led to downregulation of membrane GLAST, GLT-1, and EAAC1 in the dorsal horn of rat spinal cords, whereas etanercept inhibited the downregulation of glial GLAST and GLT-1 but not neuronal EAAC1. These data are supported by the work of Sitcheran et al.[25] who demonstrated that TNF-α induced IκB degradation, which triggered nuclear factor (NF)-κB nuclear translocation and suppressed GLT-1 expression in H4 astroglioma cells. Furthermore, increasing the TNF-α concentration leads to a reduction of GLAST protein, in a dose-dependent manner, which could be prevented by preincubation with neutralizing TNF-α antibody.[43] The authors assumed that downregulation of GLAST resulted in an excess of extracellular glutamate and triggered excitotoxicity through NMDA receptors in neuron and AMPA/kainate receptors in oligodendrocytes, and concluded that TNF-α had an important role in central nervous system inflammatory disorders via reduction of glutamate uptake. Similar to our previous study, we found that etanercept (50 μg) effectively suppressed microglia activation and proinflammatory cytokines TNF-α, interleukin (IL)-1β, and IL-6 mRNA expression.[44] Taken together with our present results, we suggest that etanercept (50 μg) treatment, via attenuation of TNF-α expression, suppresses the downregulation of glial GLAST and GLT-1 in morphine-tolerant rats. As is known, NF-κB and activator protein-1 are the most important transcriptional factors related to the TNF-α signaling pathway.[45] Central glucocorticoid receptors modulated downregulation of spinal EAAC1 after peripheral nerve injury through diminished NF-κB expression.[46] Intriguingly, we found that amitriptyline could induce GT upregulation including GLAST, GLT-1, and EAAC1 through NF-κB–dependent activation.[47] Moreover, chronic morphine exposure also induced E3 ligase activation via cyclic adenosine monophosphate/protein kinase A signaling and resulted in a ubiquitin-proteasome system mediating degradation of EAAC1.[48] This means that there are more mechanisms, besides the NF-κB pathway, involving EAAC1 regulation after chronic morphine administration, which demand more studies to elucidate the underlying signaling pathway.

In our present study, there was a significant increase in the expression of AMPA- and NMDA-receptor subunits in the spinal cords of chronic morphine-infused rats beginning on day 1 and lasting for 5 days. In contrast, Beattie et al.[26] found that TNF-α enhances synaptic strength by increasing the expression of the AMPA receptor, but not the NMDA receptor; they suggested that mobility of NMDA receptors was less than AMPA receptors because of differential attachments.[49] Compared with the brief exposure (10 minutes) of TNF-α in the study by Beattie et al., our results indicated that chronic IT morphine infusion is long enough to enhance the surface expression of both AMPA and NMDA receptors, which contributes to the reduction of morphine's antinociceptive effect. Our study confirmed that NMDA receptors are important for neuronal plasticity and spinal morphine tolerance development. Stellwagen et al.[50] also demonstrated that TNF-α preferentially increased the synaptic expression of GluR2-lacking AMPA receptors, which are more permeable to Ca^{2+} and simultaneously downregulate surface $GABA_A$ receptors, thus resulting in a reduction of inhibitory synaptic transmission. These results are consistent with our results, which showed that the GluR1 surface expression is apparently augmented compared with GluR2 subunits. Induction of GluR1 expression of AMPA receptors by TNF-α increases the neurons' vulnerability to excitotoxicity, which is mediated through acid sphingomyelinase and the NF-κB pathway.[51] GluR2 expression affected calcium permeability through AMPA receptors, and the increase of GluR2-lacking AMPA receptors will alter the function of the synaptic AMPA receptors.[52] Our results imply that TNF-α not only influences AMPA receptor expression but also modulates the amount of GluR2-abundant AMPA receptors, which control Ca^{2+} influx. NMDA receptors are highly permeable to Ca^{2+}, and the NR1 subunit of the NMDA receptor is an essential functional unit of the NMDA receptor.[53] As mentioned, NMDA receptors are involved in the mechanism of morphine tolerance and NMDA receptor antagonists have been demonstrated to inhibit morphine tolerance development.[13,16,18] Lim et al.[54] further observed an increase of NMDA receptor NR1 subunit expression in chronic morphine-infused rats' spinal cord dorsal horn, which was mediated by glucocorticoid receptor activation in a time-dependent manner. Shimoyama et al.[55] demonstrated that morphine tolerance was attenuated by deletion of the NMDA receptor NR1 subunit via IT administration of antisense oligonucleotide. Similarly, we also found that etanercept attenuated the upregulation of the NMDA receptor NR1 subunit expression in chronic morphine-infused rats. We suggested that this increase of glutamate availability by TNF-α was attributable to downregulation of membrane GTs, in accordance with upregulation of surface AMPA and NMDA receptors in chronic morphine-infused rats, which contributes to the excessive activation of glutamatergic receptors and then reduces the antinociceptive effect of morphine. Conceivably, activation of the glutamatergic receptor system will enhance the intracellular mechanisms of morphine tolerance, such as interaction with μ-opioid receptors through Ca^{2+} influx, which activate protein kinase C, nitric oxide synthase, and relevant gene regulation.[56] The glutamate-mediated excitotoxicity with excessive influx of calcium ions was proposed to be vital in neuropathic pain.[56] We proposed that the restoration of the antinociceptive effect of morphine by etanercept (50 μg) pretreatment in chronic morphine-infused rats occurs by inhibiting the TNF-α–mediated glutamatergic

synaptic transmission. In contrast to the expression of GTs, the membrane preparation cannot differentiate the neurons and glial cells and, therefore, we could not discriminate the role of astroglial AMPA and NMDA receptors in the antinociceptive effect of morphine in morphine-tolerant rats. A recent study has implied that amitriptyline preserved morphine analgesia because of inhibition of toll-like receptor 4 signaling and resulted in diminishing production of proinflammatory cytokines.[57] Our previous study also demonstrated that etanercept attenuated the proinflammatory cytokine TNF-α, IL-1β, and IL-6 mRNAs expression.[44] These results were consistent, but further analysis of the underlying molecular mechanisms of etanercept is needed to elucidate the interaction between TNF-α and morphine tolerance.

In summary, our results indicate that TNF-α has an important role in morphine tolerance, and etanercept pretreatment might diminish TNF-α signaling and partially restore the antinociceptive effect of morphine in chronic morphine-infused rats. Moreover, we found that enhancement of synaptic strength is the result of the increase of glutamate availability and expression of AMPA and NMDA receptors. Etanercept preserves the antinociceptive effect of morphine by inhibiting glutamatergic synaptic transmission. Based on the present study, we suggest that etanercept may be clinically useful in the management of patients who experience chronic pain and need long-term morphine therapy.

DISCLOSURES

Name: Ching-Hui Shen, MD.
Contribution: Participated in study design and conduct of the study, and wrote the manuscript.
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Contribution: Conduct of study and data analysis.
Name: Yueh-Hwa Tai, PhD.
Contribution: Conduct of study and data analysis.
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Name: Chih-Cheng Chien, MD, PhD.
Contribution: Manuscript review.
Name: Chih-Shung Wong, MD, PhD.
Contribution: Study design, data analysis, and manuscript revision.

REFERENCES

1. Duttaroy A, Yoburn BC. The effect of intrinsic efficacy on opioid tolerance. Anesthesiology 1995;82:1226–36
2. Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. Science 1988;242:715–23
3. Dalton GD, Smith FL, Smith PA, Dewey WL. Alterations in brain protein kinase A activity and reversal of morphine tolerance by two fragments of native protein kinase A inhibitor peptide (PKI). Neuropharmacology 2005;48:648–57
4. Wang Z, Sadee W. Tolerance to morphine at the mu-opioid receptor differentially induced by cAMP-dependent protein kinase activation and morphine. Eur J Pharmacol 2000; 389:165–71
5. Smith FL, Javed RR, Elzey MJ, Dewey WL. The expression of a high level of morphine antinociceptive tolerance in mice involves both PKC and PKA. Brain Res 2003;985:78–88
6. Tao PL, Chang LR, Chou YP, Law PY, Loh HH. Chronic opioid treatment may uncouple opioid receptors and G-proteins: evidence from radiation inactivation analysis. Eur J Pharmacol 1993;246:233–8
7. Chakrabarti S, Oppermann M, Gintzler AR. Chronic morphine induces the concomitant phosphorylation and altered association of multiple signaling proteins: a novel mechanism for modulating cell signaling. Proc Natl Acad Sci USA 2001; 98:4209–14
8. Bohn LM, Lefkowitz RJ, Caron MG. Differential mechanisms of morphine antinociceptive tolerance revealed in (beta)arrestin-2 knock-out mice. J Neurosci 2002;22:10494–500
9. Bohn LM, Gainetdinov RR, Lin FT, Lefkowitz RJ, Caron MG. Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. Nature 2000;408:720–3
10. Bohn LM, Lefkowitz RJ, Gainetdinov RR, Peppel K, Caron MG, Lin FT. Enhanced morphine analgesia in mice lacking beta-arrestin 2. Science 1999;286:2495–8
11. He L, Fong J, von Zastrow M, Whistler JL. Regulation of opioid receptor trafficking and morphine tolerance by receptor oligomerization. Cell 2002;108:271–82
12. Jordan BA, Devi LA. G-protein-coupled receptor heterodimerization modulates receptor function. Nature 1999; 399:697–700
13. Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 1991;251:85–7
14. Mao J, Price DD, Mayer DJ. Experimental mononeuropathy reduces the antinociceptive effects of morphine: implications for common intracellular mechanisms involved in morphine tolerance and neuropathic pain. Pain 1995;61:353–64
15. Law PY, Wong YH, Loh HH. Molecular mechanisms and regulation of opioid receptor signaling. Annu Rev Pharmacol Toxicol 2000;40:389–430
16. Xu NJ, Bao L, Fan HP, Bao GB, Pu L, Lu YJ, Wu CF, Zhang X, Pei G. Morphine withdrawal increases glutamate uptake and surface expression of glutamate transporter GLT1 at hippocampal synapses. J Neurosci 2003;23:4775–84
17. Wen ZH, Wu GJ, Chang YC, Wang JJ, Wong CS. Dexamethasone modulates the development of morphine tolerance and expression of glutamate transporters in rats. Neuroscience 2005;133:807–17
18. Mao J, Sung B, Ji RR, Lim G. Chronic morphine induces downregulation of spinal glutamate transporters: implications in morphine tolerance and abnormal pain sensitivity. J Neurosci 2002;22:8312–23
19. Tai YH, Wang YH, Wang JJ, Tao PL, Tung CS, Wong CS. Amitriptyline suppresses neuroinflammation and up-regulates glutamate transporters in morphine-tolerant rats. Pain 2006;124:77–86
20. Raghavendra V, Rutkowski MD, DeLeo JA. The role of spinal neuroimmune activation in morphine tolerance/hyperalgesia in neuropathic and sham-operated rats. J Neurosci 2002;22:9980–9
21. Benveniste EN, Benos DJ. TNF-alpha- and IFN-gamma-mediated signal transduction pathways: effects on glial cell gene expression and function. FASEB J 1995;9:1577–84
22. Pan W, Zadina JE, Harlan RE, Weber JT, Banks WA, Kastin AJ. Tumor necrosis factor-alpha: a neuromodulator in the CNS. Neurosci Biobehav Rev 1997;21:603–13
23. Chun J. Selected comparison of immune and nervous system development. Adv Immunol 2001;77:297–322
24. Morganti-Kossman MC, Lenzlinger PM, Hans V, Stahel P, Csuka E, Ammann E, Stocker R, Trentz O, Kossmann T. Production of cytokines following brain injury: beneficial and deleterious for the damaged tissue. Mol Psychiatry 1997; 2:133–6
25. Sitcheran R, Gupta P, Fisher PB, Baldwin AS. Positive and negative regulation of EAAT2 by NF-kappaB: a role for N-myc in TNFalpha-controlled repression. EMBO J 2005;24:510–20
26. Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC. Control of synaptic strength by glial TNFalpha. Science 2002;295:2282–5
27. Grassi F, Mileo AM, Monaco L, Punturieri A, Santoni A, Eusebi F. TNF-alpha increases the frequency of spontaneous miniature synaptic currents in cultured rat hippocampal neurons. Brain Res 1994;659:226–30

28. Emch GS, Hermann GE, Rogers RC. TNF-alpha activates solitary nucleus neurons responsive to gastric distension. Am J Physiol Gastrointest Liver Physiol 2000;279:G582–6
29. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998;41:1552–63
30. Scott DL, Kingsley GH. Tumor necrosis factor inhibitors for rheumatoid arthritis. N Engl J Med 2006;355:704–12
31. Marzo-Ortega H, McGonagle D, O'Connor P, Emery P. Efficacy of etanercept in the treatment of the entheseal pathology in resistant spondylarthropathy: a clinical and magnetic resonance imaging study. Arthritis Rheum 2001;44:2112–7
32. Wilson JA. Tumor necrosis factor alpha and colitis-associated colon cancer. N Engl J Med 2008;358:2733–4
33. Tobinick EL, Gross H. Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. J Neuroinflammation 2008;5:2
34. Grossman ML, Basbaum AI, Fields HL. Afferent and efferent connections of the rat tail flick reflex (a model used to analyze pain control mechanisms). J Comp Neurol 1982;206:9–16
35. Tai YH, Wang YH, Tsai RY, Wang JJ, Tao PL, Liu TM, Wang YC, Wong CS. Amitriptyline preserves morphine's antinociceptive effect by regulating the glutamate transporter GLAST and GLT-1 trafficking and excitatory amino acids concentration in morphine-tolerant rats. Pain 2007;129:343–54
36. Mao J, Price DD, Mayer DJ. Thermal hyperalgesia in association with the development of morphine tolerance in rats: roles of excitatory amino acid receptors and protein kinase C. J Neurosci 1994;14:2301–12
37. Wong CS, Chang YC, Yeh CC, Huang GS, Cherng CH. Loss of intrathecal morphine analgesia in terminal cancer patients is associated with high levels of excitatory amino acids in the CSF. Can J Anaesth 2002;49:561–5
38. Kaul M, Garden GA, Lipton SA. Pathways to neuronal injury and apoptosis in HIV-associated dementia. Nature 2001;410:988–94
39. Bennett GJ. Update on the neurophysiology of pain transmission and modulation: focus on the NMDA-receptor. J Pain Symptom Manage 2000;19:S2–6
40. Danbolt NC. Glutamate uptake. Prog Neurobiol 2001;65:1–105
41. Schlag BD, Vondrasek JR, Munir M, Kalandadze A, Zelenaia OA, Rothstein JD, Robinson MB. Regulation of the glial Na+-dependent glutamate transporters by cyclic AMP analogs and neurons. Mol Pharmacol 1998;53:355–69
42. Wen ZH, Chang YC, Cherng CH, Wang JJ, Tao PL, Wong CS. Increasing of intrathecal CSF excitatory amino acids concentration following morphine challenge in morphine-tolerant rats. Brain Res 2004;995:253–9
43. Korn T, Magnus T, Jung S. Autoantigen specific T cells inhibit glutamate uptake in astrocytes by decreasing expression of astrocytic glutamate transporter GLAST: a mechanism mediated by tumor necrosis factor-alpha. FASEB J 2005;19:1878–80
44. Shen CH, Tsai RY, Shih MS, Lin SL, Tai YH, Chien CC, Wong CS. Etanercept restores the antinociceptive effect of morphine and suppresses spinal neuroinflammation in morphine-tolerant rats. Anesth Analg 2011;112:454–9
45. Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol 2001;11:372–7
46. Wang S, Lim G, Yang L, Sung B, Mao J. Downregulation of spinal glutamate transporter EAAC1 following nerve injury is regulated by central glucocorticoid receptors in rats. Pain 2006;120:78–85
47. Tai YH, Tsai RY, Wang YH, Cherng CH, Tao PL, Liu TM, Wong CS. Amitriptyline induces nuclear transcription factor-kappaB-dependent glutamate transporter upregulation in chronic morphine-infused rats. Neuroscience 2008;153:823–31
48. Yang L, Wang S, Sung B, Lim G, Mao J. Morphine induces ubiquitin-proteasome activity and glutamate transporter degradation. J Biol Chem 2008;283:21703–13
49. Allison DW, Gelfand VI, Spector I, Craig AM. Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. J Neurosci 1998;18:2423–36
50. Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. J Neurosci 2005;25:3219–28
51. Yu Z, Cheng G, Wen X, Wu GD, Lee WT, Pleasure D. Tumor necrosis factor alpha increases neuronal vulnerability to excitotoxic necrosis by inducing expression of the AMPA-glutamate receptor subunit GluR1 via an acid sphingomyelinase- and NF-kappaB-dependent mechanism. Neurobiol Dis 2002;11:199–213
52. Washburn MS, Numberger M, Zhang S, Dingledine R. Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. J Neurosci 1997;17:9393–406
53. Forrest D, Yuzaki M, Soares HD, Ng L, Luk DC, Sheng M, Stewart CL, Morgan JI, Connor JA, Curran T. Targeted disruption of NMDA receptor 1 gene abolishes NMDA response and results in neonatal death. Neuron 1994;13:325–38
54. Lim G, Wang S, Zeng Q, Sung B, Yang L, Mao J. Expression of spinal NMDA receptor and PKCgamma after chronic morphine is regulated by spinal glucocorticoid receptor. J Neurosci 2005;25:11145–54
55. Shimoyama N, Shimoyama M, Davis AM, Monaghan DT, Inturrisi CE. An antisense oligonucleotide to the N-methyl-D-aspartate (NMDA) subunit NMDAR1 attenuates NMDA-induced nociception, hyperalgesia, and morphine tolerance. J Pharmacol Exp Ther 2005;312:834–40
56. Mao J, Price DD, Mayer DJ. Mechanisms of hyperalgesia and morphine tolerance: a current view of their possible interactions. Pain 1995;62:259–74
57. Hutchinson MR, Loram LC, Zhang Y, Shridhar M, Rezvani N, Berkelhammer D, Phipps S, Foster PS, Landgraf K, Falke JJ, Rice KC, Maier SF, Yin H, Watkins LR. Evidence that tricyclic small molecules may possess toll-like receptor and myeloid differentiation protein 2 activity. Neuroscience 2010;168:551–63