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Intrathecal RGS4 Inhibitor, CCG50014, Reduces Nociceptive Responses and Enhances Opioid-Mediated Analgesic Effects in the Mouse Formalin Test

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BACKGROUND: The regulator of G-protein signaling protein type 4 (RGS4) accelerates the guanosine triphosphatase activity of $G_{\alpha i}$ and $G_{\alpha o}$, resulting in the inactivation of G-protein–coupled receptor signaling. An opioid receptor (OR), a $G_{\alpha i}$-coupled receptor, plays an important role in pain modulation in the central nervous system. In this study, we examined whether (1) spinal RGS4 affected nociceptive responses in the formalin pain test, (2) this RGS4-mediated effect was involved in OR activation, and (3) the μ-OR agonist–induced antinociceptive effect was modified by RGS4 modulation.

METHODS: Formalin (1%, 20 μL) was injected subcutaneously into the right hindpaws of male 129S4/SvJae×C57BL/6J (*RGS4*$^{+/+}$ or *RGS4*$^{-/-}$) mice, and the licking responses were counted for 40 minutes. The time periods (seconds) spent licking the injected paw during 0 to 10 minutes (early phase) and 10 to 40 minutes (late phase) were measured as indicators of acute nociception and inflammatory pain response, respectively. An RGS4 inhibitor, CCG50014, and/or a μ-OR agonist, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO), were intrathecally injected 5 minutes before the formalin injection. A nonselective OR antagonist, naloxone, was intraperitoneally injected 30 minutes before the CCG50014 injection.

RESULTS: Mice that received the formalin injection exhibited typical biphasic nociceptive behaviors. The nociceptive responses in RGS4-knockout mice were significantly decreased during the late phase but not during the early phase. Similarly, intrathecally administered CCG50014 (10, 30, or 100 nmol) attenuated the nociceptive responses during the late phase in a dose-dependent manner. The antinociceptive effect of the RGS4 inhibitor was totally blocked by naloxone (5 mg/kg). In contrast, intrathecal injection of DAMGO achieved a dose-dependent reduction of the nociceptive responses at the early and late phases. This analgesic effect of DAMGO was significantly enhanced by the genetic depletion of RGS4 or by coadministration of CCG50014 (10 nmol).

CONCLUSIONS: These findings demonstrated that spinal RGS4 inhibited the endogenous or exogenous OR-mediated antinociceptive effect in the formalin pain test. Thus, the inhibition of RGS4 activity can enhance OR agonist–induced analgesia. The enhancement of OR agonist–induced analgesia by coadministration of the RGS4 inhibitor suggests a new therapeutic strategy for the management of inflammatory pain. (Anesth Analg 2015;120:671–7)

G-protein–coupled receptor (GPCR) signaling is initiated by agonist-induced receptor activation, which converts the trimeric $G_{\alpha\beta\gamma}$ complex into separate G_{α} and $G_{\beta\gamma}$ subunits. The G_{α} subunit initially is bound to guanosine diphosphate coupled to the inactive receptor. Upon receptor activation, the G_{α} subunit converts to an active guanosine triphosphate–bound state, promoting signal transduction.[1–3] GPCRs do not act alone; several accessory proteins modulate the activities of GPCRs. One important group of accessory proteins is the regulators of G-protein signaling (RGS) protein family.[4,5] RGS proteins are guanosine triphosphatase (GTPase)-activating proteins that accelerate signaling termination. RGS proteins are a family of cellular proteins that contain a homologous RGS domain of approximately 120 amino acids in length. More than 20 different mammalian RGS genes have been described. These genes are grouped into 9 classes based on sequence similarities and common structural features outside the RGS domain.[5]

Each type of RGS protein exhibits distinct selectivity and specificity in its regulation of receptors.[6] RGS4 is a member of the R4 subfamily of RGS proteins and has a structure consisting of the RGS homology domain and a small N-terminus. RGS4 primarily regulates the $G_{\alpha i/o}$ protein and not the $G_{\alpha s}$ protein.[5,6] RGS4 is widely expressed in the central nervous system.[7–9] Thus, RGS4 is a potential regulator of neurotransmission through GPCRs, including opioid, noradrenergic, dopaminergic, or serotonergic signals, because of reduced duration of ligand-based receptor signaling.[10–14]

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The opioid receptor (OR) plays an important role in the regulation of nociceptive mechanisms in the peripheral and central nervous system.[15] The OR is fundamentally related to the $G_{\alpha i/o}$ class of adenylate cyclase–inhibitory proteins; thus, activation of these receptors by agonists ameliorates pain.[16,17] OR agonists, such as morphine, generally are used as potent analgesic drugs. RGS4 blunts the abilities of μ- or δ-OR agonists to inhibit cyclic adenosine 3′,5′-monophosphate (cAMP) accumulation in various cell lines.[12,18,19] However, it is unclear whether RGS4 is involved in acute pain signaling, and no clinical trials have tested RGS4 modulators for the management of pain.

The present study was designed to examine the potential role of RGS4 in acute inflammatory pain. RGS4 generally is distributed in multiple cells throughout the body. We focused on RGS4 in the spinal cord because this is a crucial converging point for various painful signals from peripheral sites to the higher brainstem. RGS4 and ORs are highly expressed in the spinal dorsal horn. We examined (1) the effects of spinal RGS4 on nociceptive responses in the formalin pain test using RGS4-knockout (RGS4KO) mice or intrathecal injection of the RGS4 inhibitor 4-[(4-fluorophenyl)methyl]-2-(4-methylphenyl)-1,2,4-thiadiazolidine-3,5-dione (CCG50014), (2) the involvement of the RGS4-mediated antinociceptive effect in OR activation, and (3) potential modification of the antinociceptive effect induced by the μ-OR agonist, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO) by genetic depletion of RGS4, or the RGS4 inhibitor CCG50014.

METHODS

Animals

The mice we used had a genetic background of 129S4/SvJae×C57BL/6J. Adult male *RGS4*$^{-/-}$, *RGS4*$^{+/-}$, and wild-type littermate mice (25–30 g) of the B6×129 F1 hybrid were obtained by mating the parental strains C57BL/6J *RGS4*$^{+/-}$ and 129S4/SvJae *RGS4*$^{+/-}$. All experimental animals were obtained from the Laboratory Animal Center of the Korea Institute of Science and Technology in Korea. They were housed in colony cages with free access to food and water and maintained in temperature-controlled and light-controlled rooms (22 ± 2°C, 12/12-hour light/dark cycle with lights on at 8:00 AM) for at least 1 week before the study. All the methods used in the present study were approved by the Institutional Animal Care and Use Committee of Korea Institute of Science and Technology and conform to National Institutes of Health guidelines (NIH publication No. 86-23, revised 1985). All algesiometric assays were conducted in accordance with the ethical guidelines established by the International Association for the Study of Pain.

Drugs

CCG50014 (RGS4 inhibitor) and DAMGO (μ-OR agonist) were purchased from Tocris (Bristol, United Kingdom). Naloxone (nonspecific OR antagonist) was purchased from Sigma (St. Louis, MO). CCG50014 was diluted in 12% dimethyl sulfoxide (DMSO) in saline. DAMGO and naloxone were diluted in saline.

Intrathecal Drug Treatment

Each drug was administered by intrathecal injection based on the technique developed by Hylden and Wilcox.[20] Drugs were dissolved in 5 μL of vehicle. We injected a 5-μL volume intrathecally because data suggest that this is likely to be the upper limit that can be reliably injected into a mouse without appreciable redistribution of the drug through the cerebrospinal fluid to the basal cisterns of the brain. In brief, for mouse intrathecal injections, a 30-gauge needle (length: 0.5 inch) connected to a 50-μL Hamilton syringe (Hamilton, Reno, NV) was inserted into the subarachnoid space between the lumbar vertebrae L5 and L6. A flick of the mouse's tail provided a reliable indicator that the needle had penetrated the dura mater. The syringe was held in position for a few seconds after the injection of 5 μL/mouse.

Formalin-Induced Pain Behaviors

Mice were first acclimatized for 30 minutes in an acrylic observation chamber (size ranges 12 × 12× 12 cm); 20 μL of 1% formalin was then injected subcutaneously into the plantar surface of the right hindpaw with a 30-gauge needle, as previously described.[21] After injection of formalin, mice were immediately placed in a test chamber; nociceptive responses were digitally videotaped from underneath a glass floor for 40 minutes. The summation of time (in seconds) spent licking and biting the formalin-injected hindpaw during each 5-minute block was measured as an indicator of nociception. The duration of the responses during the first 10-minute period represented the early phase, whereas the duration of responses during the subsequent 30-minute period (from 10 to 40 minutes after injection) represented the late phase of the formalin test. In this experiment, CCG50014 (10, 30, or 100 nmol) or DAMGO (0.03, 0.1, 0.3, 1, 3, 10, 30, or 100 pmol) was intrathecally injected 5 minutes before the formalin injection. Naloxone (5 mg/kg) was administered intraperitoneally 30 minutes before intrathecal administration of CCG50014 (100 nmol). The dose of naloxone was selected based on previously published work.[22–24]

Statistical Analysis

Sample size was estimated based on the "resource equation" method for the first experiment (Fig. 1) to see whether RGS4 knockout affected the pain responses (7–10 animals). After observing the effect of RGS4 on nociceptive behavioral responses, the sample size for other experiments was estimated by power analysis using G*power 3.1 (Faul, University of Kiel, Kiel, Germany) with power = 0.8 and alpha = 0.05 based on the data from our previous studies[25,26] (5–7 animals depending on animal availability). Data are presented as the mean ± SEM. Statistical analysis was performed using Prism 6.0 (GraphPad Software, San Diego, CA). Data were analyzed using 1-way or 2-way analysis of variance (ANOVA), followed by Tukey post hoc test for multiple comparisons (Tukey-corrected P value). Before running the ANOVA test, we performed residual analysis to assure that the assumptions of normal distribution and equal variance were met. In the present study, the residuals of each ANOVA were normally distributed (Lilliefors test, $P > 0.2$) and had equal variances among groups (Levene mean-based test, $P > 0.05$). Dose-response curve fitting and 50% effective dose (ED_{50}) determinations were performed using the variable slope sigmoidal dose-response analysis tool in Prism. The dose-response curves were compared using the

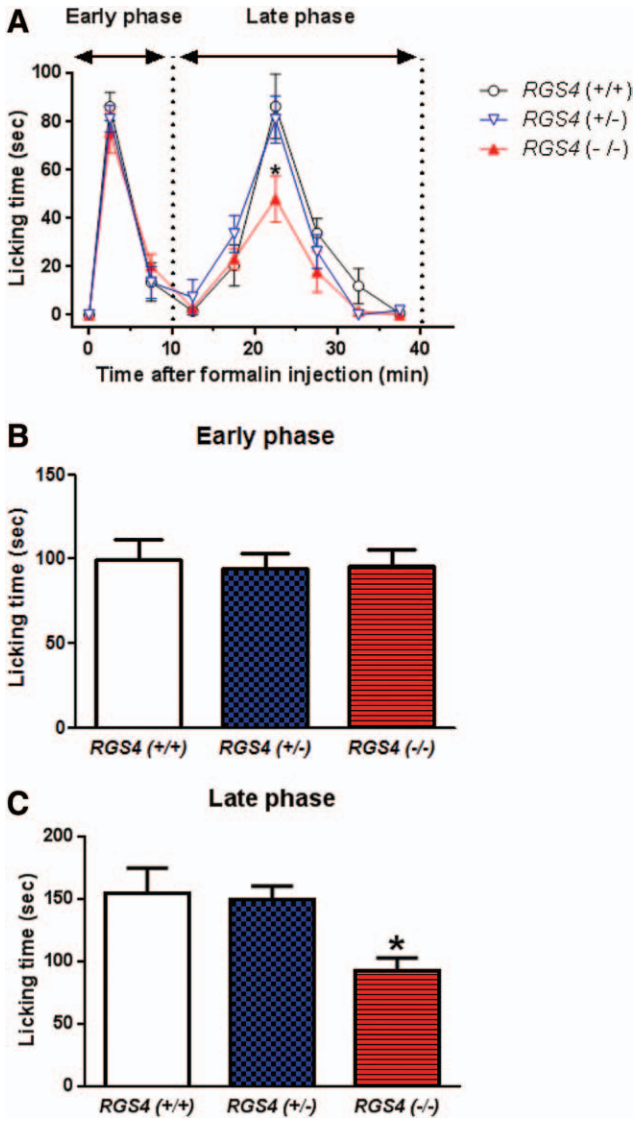

Figure 1. Effect of genetic depletion of regulator of G-protein signaling protein type 4 (RGS4) on formalin-induced nociceptive responses. Intraplantar formalin injection showed biphasic nociceptive responses (early phase: 0–10 minutes and late phase: 10–40 minutes) in RGS4-knockout (RGS4KO, *RGS*$^{-/-}$), RGS4-heterozygous (*RGS4*$^{+/-}$), and wild-type mice groups (A). RGS4KO mice showed reduced nociceptive responses in the late phase (C) but not the early phase (B). Tukey post hoc test *$P < 0.01$, significantly different from the value in the wild-type group; $n = 7$–10 mice per group.

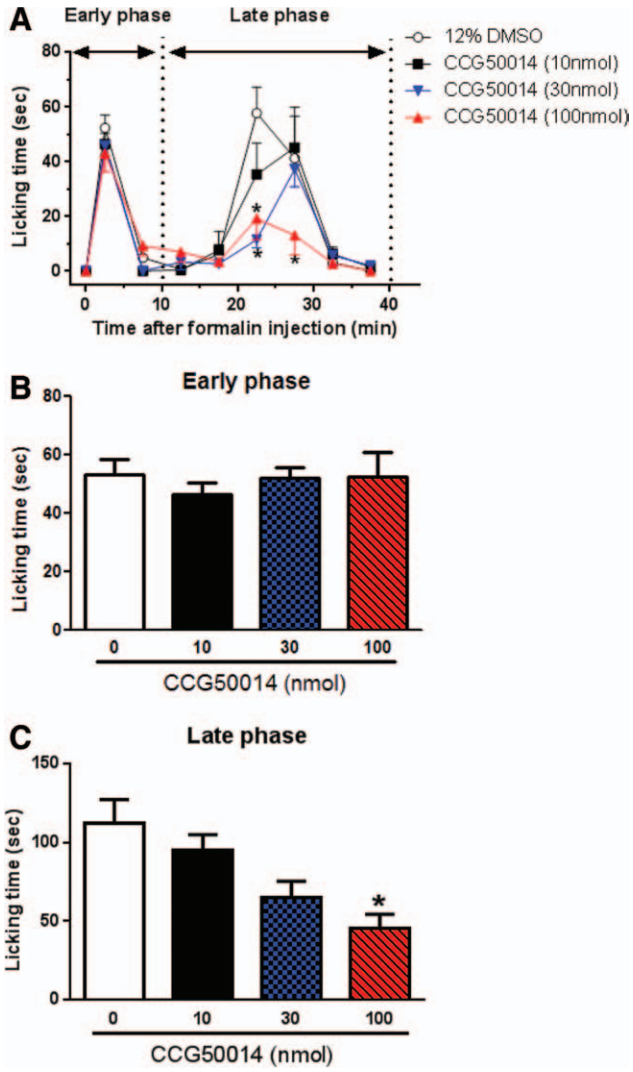

Figure 2. Effect of intrathecal 4-[(4-fluorophenyl)methyl]-2-(4-methylphenyl)-1,2,4-thiadiazolidine-3,5-dione (CCG50014) in the formalin-induced nociceptive response. Vehicle (12% dimethyl sulfoxide [DMSO]) or CCG50014 (10, 30, or 100 nmol) was intrathecally injected 5 minutes before the formalin injection. CCG50014 suppressed the formalin-induced nociceptive responses in a dose-dependent manner in the late phase (A and C), but not in the early phase (A and B). Tukey post hoc test *$P < 0.01$, significantly different from the value in the vehicle group; $n = 7$ mice per group.

extra sum-of-squares F test to determine whether the data represented distinct curves between treatments. We considered a P value of <0.01 to be statistically significant. When P value was between 0.01 and 0.15, results from analyses were reported with corresponding 95% confidence intervals (CIs).

RESULTS

Effect of Genetic Depletion or Pharmacologic Inhibition of RGS4 on Formalin-Induced Nociceptive Responses

Intraplantar injection of formalin into the hindpaw of a mouse produced the typical biphasic nociceptive responses consisting of an early phase (0–10 minutes) followed by a late, prolonged phase (10–40 minutes) (Fig. 1A and Fig. 2A). RGS4KO mice showed normal acute nociceptive responses during the early phase (Fig. 1B; $P = 0.91$) but reduced responses during the late phase compared with that shown by the wild-type mice (Fig. 1A; $P < 0.0001$ at 20–25-minute block; and Fig. 1C; $P = 0.0051$). RGS4 heterozygous and wild-type mice did not show significant differences in both phases of the formalin test (Fig. 1B; $P = 0.91$ in early phase and $P = 0.96$ in late phase). Similar to RGS4KO, intrathecal administration of the RGS4 inhibitor, CCG50014 (10, 30, or 100 nmol), showed a dose-dependent analgesic effect on formalin-induced nociceptive responses during the late phase (Fig. 2A, $P < 0.0001$ for vehicle versus 100 nmol at 20–25- and 25–30-minute block, $P < 0.0001$ for vehicle versus 30 nmol at 20–25-minute block; Fig. 2C, $P = 0.0019$ for vehicle versus 100 nmol) but not during the early phase (Fig. 2B, $P = 0.84$ for vehicle versus 10 nmol,

P = 0.95 for vehicle versus 30 nmol, P = 1.00 for vehicle versus 100 nmol). Intrathecal CCG50014, however, did not affect the reduced nociceptive response of RGS4KO mice in the late phase (Supplemental Digital Content, Figure, http://links.lww.com/AA/B61, P = 0.0005 for vehicle/wild versus CCG50014/wild, P = 0.0093 for vehicle/wild versus vehicle/RGS4KO, P = 0.0034 for vehicle/wild versus CCG50014/RGS4KO, and P = 0.83 for vehicle/RGS4KO versus CCG50014/RGS4KO).

Effect of Spinal OR Antagonist on RGS4 Inhibitor–Induced Antinociception

Subsequently, we tested whether ORs were involved in RGS4-mediated analgesic effects by using the nonselective OR antagonist, naloxone. Intraperitoneal administration of naloxone (5 mg/kg) alone did not affect formalin-induced pain behavior during the early (Fig. 3A; P = 1.00) or late phase (Fig. 3B; P = 0.98) compared with that of vehicle control. However, the analgesic effect of CCG50014 (P < 0.0001 for 12% DMSO plus saline versus CCG plus saline) during the late phase was completely blocked by pretreatment with naloxone (Fig. 3B; P = 0.0001 for CCG plus saline versus CCG plus naloxone).

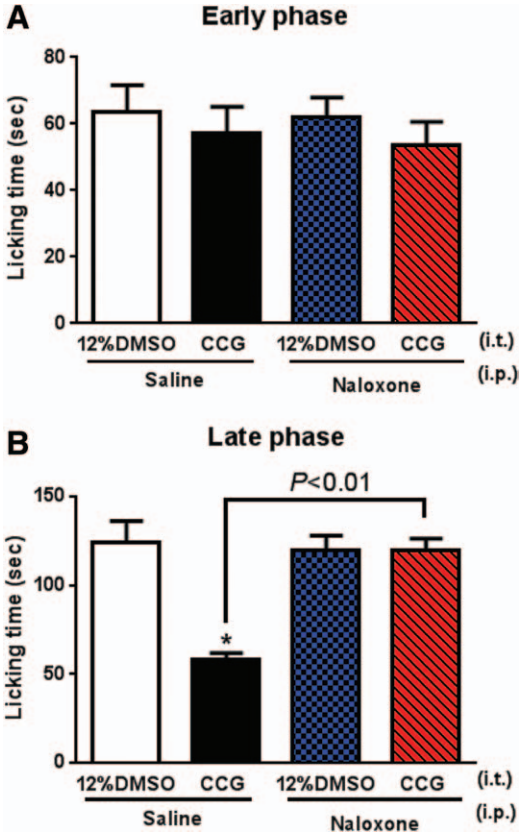

Figure 3. Effect of the nonselective opioid receptor antagonist, naloxone, on 4-[(4-fluorophenyl)methyl]-2-(4-methylphenyl)-1,2,4-thiadiazolidine-3,5-dione (CCG50014)-induced antinociceptive effect during the early (A) and late phases (B) in the formalin test. Naloxone (5 mg/kg) or saline was intraperitoneally injected 30 minutes before intrathecal injection of CCG50014 (100 nmol) or 12% dimethyl sulfoxide (DMSO). Naloxone totally blocked CCG50014-induced antinociceptive effects in the late phase (B). Tukey post hoc test *P < 0.01, significantly different from the value in the intraperitoneal saline and intrathecal 12% DMSO-treated group; n = 7 mice per group.

Effect of RGS4 Inhibition on Exogenous DAMGO-Induced Antinociception

To test whether inhibition of RGS4 affected OR agonist–induced analgesic effects, the μ-OR agonist, DAMGO (1, 10, or 100 pmol), was intrathecally administered in RGS4KO mice. Treatment of DAMGO alone showed dose-dependent suppression of the nociceptive responses during the early (Fig. 4A, P < 0.0001 for vehicle versus 100 pmol at 0–5-minute block; Fig. 4B, P < 0.0001 for vehicle versus 100 pmol, 1 vs 100 pmol, and 10 vs 100 pmol) and late phase (Fig. 4A, P < 0.0001 for vehicle versus 100 pmol at 20–25- and 25–30-minute block, P < 0.0001 at 20–25-minute block for vehicle versus 10 pmol; Fig. 4C, P < 0.0001 for vehicle versus 100 pmol, P = 0.0016 for vehicle versus 10 pmol, P = 0.0035 for 1 vs 10 pmol, and P = 0.0077 for 10 vs 100 pmol). Interestingly, the dose-response curve of DAMGO was shifted to the left in RGS4KO mice compared with that in the wild-type mice in both the early phase (Fig. 5A; ED_{50}: DAMGO in wild-type mice = 19.78 pmol, 95% CI, 13.90–28.16 pmol versus DAMGO in RGS4KO = 1.34 pmol, 95% CI, 0.91–1.99 pmol; P = 0.0089 for 10 pmol/wild, P = 0.0003 for 30 pmol/wild, P < 0.0001 for 100 pmol/wild, P = 0.0085 for 1 pmol/RGS4KO, P < 0.0001 for 3 pmol/RGS4KO, and P < 0.0001 for 10 pmol/RGS4KO

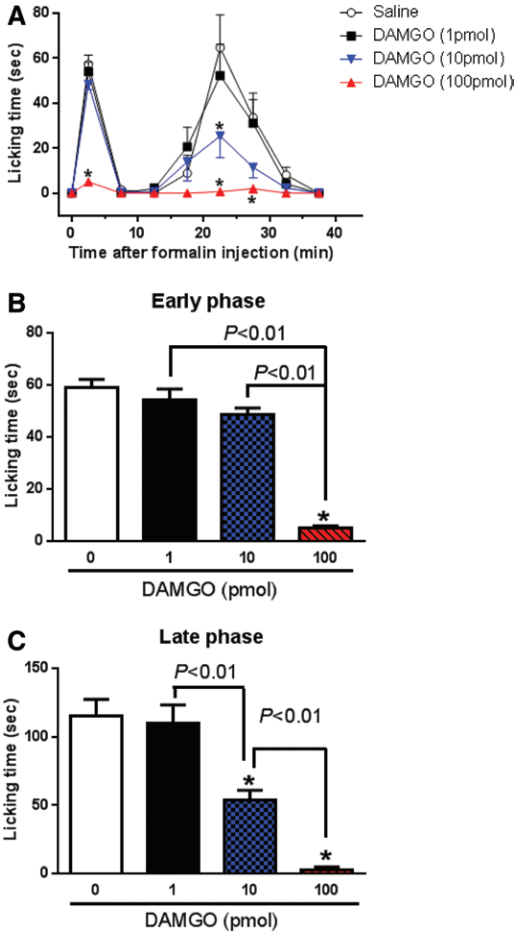

Figure 4. Effect of intrathecal treatment of [D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin (DAMGO) in the formalin test. DAMGO suppressed formalin-induced nociceptive responses in a dose-dependent manner in both the early (A and B) and late phases (A and C). Tukey post hoc test *P < 0.01, significantly different from the value in the saline-treated group; n = 5 mice per group.

versus vehicle/wild type) and the late phase (Fig. 5B; ED_{50}: DAMGO in wild-type mice = 10.55 pmol, 95% CI, 7.92–14.06 pmol versus DAMGO in RGS4KO = 0.54 pmol, 95% CI, 0.38–0.76 pmol; $P < 0.0001$ for 10 pmol/wild, $P < 0.0001$ for 30 pmol/wild, $P < 0.0001$ for 100 pmol/wild, $P = 0.0031$ for vehicle/RGS4KO, $P = 0.0002$ for 0.1 pmol/RGS4KO, $P < 0.0001$ for 1 pmol/RGS4KO, $P < 0.0001$ for 3 pmol/RGS4KO, $P < 0.0001$ for 10 pmol/RGS4KO versus vehicle/wild type). The dose-response curve of DAMGO was statistically different between the RGS4KO and wild-type mice groups (extra sum-of-squares F test, $P < 0.0001$). Moreover, to verify whether pharmacologic inhibition of RGS4 affected OR agonist–induced analgesic effects, DAMGO was coadministered intrathecally with a subeffective dose of CCG50014 (10 nmol). Coadministration of DAMGO and CCG50014 (10 nmol) caused leftward shifts of the median effective doses on the dose-response curves for DAMGO during the early phase (Fig. 6A; ED_{50}: DAMGO plus vehicle [12% DMSO] = 20.64 pmol, 95% CI, 13.21–32.24 pmol versus DAMGO plus CCG50014 = 1.22 pmol, 95% CI, 0.78–1.91 pmol; $P = 0.0086$ for 30 pmol/12% DMSO, $P < 0.0001$ for 100 pmol/12% DMSO, $P = 0.0073$ for 1 pmol/CCG50014, $P < 0.0001$ for 3 pmol/CCG50014, $P < 0.0001$ for 10 pmol/CCG50014 versus 12% DMSO/saline) and the late phase (Fig. 6B; ED_{50}: DAMGO plus vehicle = 9.38 pmol, 95% CI, 6.12–14.35 pmol versus DAMGO plus CCG50014 = 0.77 pmol, 95% CI, 0.55–1.07 pmol; $P = 0.0012$ for 10 pmol/12% DMSO, $P = 0.005$ for 30 pmol/12% DMSO, $P < 0.0001$ for 100 pmol/12% DMSO, $P = 0.0068$ for 1 pmol/CCG50014, $P < 0.0001$ for 3 pmol/CCG50014, $P < 0.0001$ for 10 pmol/CCG50014 versus 12% DMSO/saline group). The dose-response curve of DAMGO was statistically different between the DAMGO plus vehicle and the DAMGO plus CCG50014 groups (extra sum-of-squares F test, $P < 0.0001$). In contrast, intrathecal injection of CCG50014 alone at 10 nmol did not show any analgesic effect in the formalin test (Fig. 6A, $P = 0.95$; Fig. 6B, $P > 0.10$).

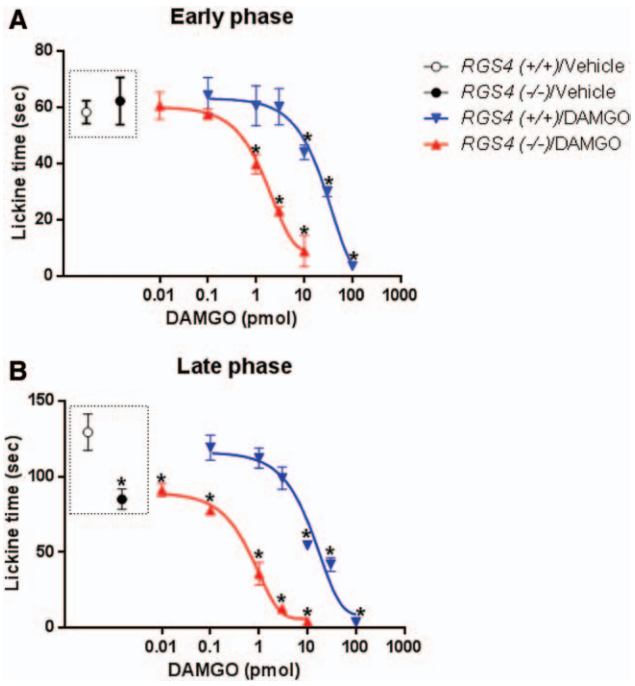

Figure 5. Dose-response curve of antinociceptive effect induced by intrathecal [D-Ala^2,NMe-Phe^4,Gly-ol^5]-enkephalin (DAMGO) in RGS4-knockout (RGS4KO) or wild-type mice during the early (A) and late (B) phases in the formalin test. Dose-response curve of DAMGO was shifted to the left in RGS4KO mice compared with that in wild control mice in the early phase (50% effective dose: DAMGO in wild-type mice = 19.78 pmol, 95% confidence intervals (CIs), 13.90–28.16 pmol versus DAMGO in RGS4KO = 1.34 pmol, 95% CI, 0.91–1.99 pmol, A) and the late phase (50% effective dose: DAMGO in mice = 10.55 pmol, CI, 7.92–14.06 pmol versus DAMGO in RGS4KO = 0.54 pmol, CI, 0.38–0.76 pmol, B). Tukey post hoc test *$P < 0.01$, significantly different from the value in vehicle (saline)-treated wild-type mice group; $n = 5$ mice per group.

DISCUSSION

RGS4 Inhibition in the Spinal Cord Reduces Nociceptive Responses During the Late Phase

RGS proteins possess specificity and selectivity in their regulation of G-protein–coupled signal transduction.[6] RGS4 selectively accelerates the GTPase activities of $G_{\alpha i}$ and $G_{\alpha o}$ but not those of $G_{\alpha s}$. In the present study, RGS4KO mice showed markedly attenuated formalin-induced nociceptive responses compared with those of wild-type or heterozygous mice (Fig. 2B). This effect was confined to the late phase of the typical biphasic pain response. When CCG50014 was injected intrathecally in the spinal cord, it also suppressed formalin-induced licking behavior during the late phase but

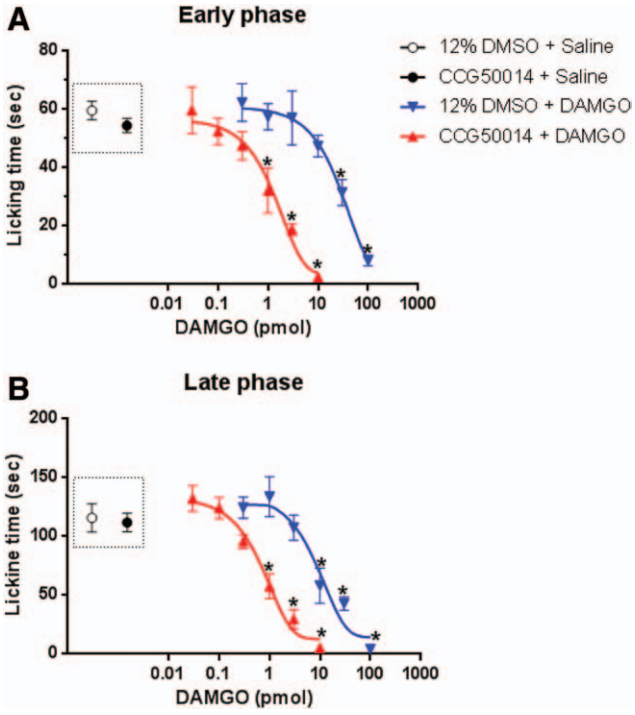

Figure 6. Dose-response curve of antinociceptive effect induced by intrathecal [D-Ala^2,NMe-Phe^4,Gly-ol^5]-enkephalin (DAMGO) alone or in combination with intrathecal 4-[(4-fluorophenyl)methyl]-2-(4-methylphenyl)-1,2,4-thiadiazolidine-3,5-dione, 10 nmol (CCG50014) during the early (A) and late (B) phases in the formalin test. The combination of DAMGO with CCG50014 resulted in a leftward shift of the dose-response curve of DAMGO in both the early phase (50% effective dose: DAMGO plus vehicle [12% dimethyl sulfoxide, DMSO] = 20.64 pmol, CI, 13.21–32.24 pmol versus DAMGO plus CCG50014 = 1.22 pmol, CI, 0.78–1.91 pmol, A) and the late phases (50% effective dose: DAMGO plus vehicle = 9.38 pmol, CI, 6.12–14.35 pmol versus DAMGO plus CCG50014 = 0.77 pmol, CI, 0.55–1.07 pmol, B). Tukey post hoc test *$P < 0.01$, significantly different from the value in 12% DMSO plus saline-treated group; $n = 5$ mice per group.

not during the early phase (Figs. 1–2). These results indicate that spinal cord RGS4 is responsible for late-phase nociceptive responses in the formalin-induced pain test. In contrast, the effect of genetic ablation of RGS4 was lesser than the effect by the 100 nmol CCG50014. This may be due to a functional readjustment of molecular signaling to compensate for the loss of RGS4 during the developmental period; pharmacologic inactivation may not normalize signaling rapidly or sufficiently enough. Because intrathecal injection of CCG50014 did not affect the nociceptive response of RGS4KO mice, it is proposed that CCG50014 mainly had an effect on RGS4 protein in this study (Supplemental Digital Content, Figure, http://links.lww.com/AA/B61). Another interesting observation was that the effect of RGS4 inhibition was confined to the late phase of the nociceptive response. Considering that the formalin-induced nociceptive responses were attributed to direct stimulation of the nociceptors in the early phase and to inflammatory and/or spinal sensitization in the late phase,[27,28] RGS4 is more likely to be involved in spinal sensitization during acute inflammatory pain.

OR Is Responsible for Spinal RGS4 Inhibition–Mediated Analgesia

ORs play a critical role in pain regulation; therefore, OR agonists have been developed as powerful analgesic drugs.[15] We examined whether RGS4 inhibitor–induced antinociception during the late phase was mediated by OR activation. A nonselective OR antagonist, naloxone, totally blocked RGS4 inhibitor–induced analgesia. This result suggests that RGS4 inhibits endogenous OR-mediated signaling. The endogenous opioid system in the central nervous system is activated when animals are exposed to painful peripheral stimuli, such as subcutaneous formalin injection. Intraplantar injection of formalin induces the release of met-enkephalin or β-endorphin from the central nervous system.[29,30] Thus, intracerebroventricular or intrathecal treatment with antiserum or an antagonist against β-endorphin or leu-enkephalin increases formalin-induced nociceptive responses in the late phase.[31,32] In addition, Zhao et al.[33] demonstrated that μ-OR knockout mice showed nociceptive responses only during the late phase, indicating that the endogenous OR exclusively affects nociceptive responses in the late phase and not during the early phase. Therefore, our data imply that spinal RGS4 inhibition–mediated analgesia during the late phase is closely associated with endogenous activation of ORs in the formalin test.

RGS4 Inhibition Potentiates Antinociception Induced by Exogenous DAMGO

We investigated the antinociceptive effect of intrathecal DAMGO in RGS4KO mice. DAMGO dose dependently suppressed nociceptive responses during the early and late phases of the formalin test. Interestingly, RGS4KO mice showed leftward shifts in dose-response curves for DAMGO-induced antinociception during the early and late phases. We subsequently examined the antinociceptive effect of intrathecal DAMGO alone or in combination with CCG50014 in the formalin test. Coadministration of DAMGO with CCG50014 produced dramatic leftward shifts in the dose-response curves for DAMGO-induced antinociception. These data indicated that RGS4 inhibition with CCG50014 enhanced the spinal μ-OR–mediated analgesic effect by increasing μ-OR activity in formalin-induced inflammatory pain.

It is interesting that either genetic depletion of RGS4 or intrathecally reduced nociceptive responses occurred only in the late phase but affected pain responses in both the early and late phases in DAMGO-induced antinociception. The endogenous opioid system primarily plays an analgesic role during the late phase.[32,33] Exogenous opioid-related drugs (i.e., DAMGO), however, can affect formalin-induced nociception in both the early and late phases. In this regard, several lines of evidence indicate that exogenous opioids induce antinociceptive effects in both phases of the formalin test.[34,35] Collectively, these studies suggest that spinal RGS4 inhibits the action of the endogenous OR system in the late phase and reduces the antinociceptive effects of an exogenous OR agonist during both the early and late phases.

Treatment with DAMGO induces robust internalization of μ-OR in HEK293 cells.[36] Several studies have also shown that RGS4 facilitates OR agonist–induced OR internalization.[12,37] Therefore, it is possible that spinal cord RGS4 inhibits the OR-mediated analgesic effect and simultaneously accelerates OR internalization. In this regard, the RGS4 inhibitor, CCG50014, is likely to enhance the DAMGO-mediated analgesic effect by removing RGS4-mediated inhibition of ORs and by preventing OR internalization in the spinal cord. The relationship between RGS4 and OR internalization in acute inflammatory pain, however, remains to be examined.

In conclusion, the present study showed the following: (1) Genetic deletion of RGS4 or intrathecal treatment with the RGS4 inhibitor, CCG50014, reduced formalin-induced pain during the late phase; (2) The CCG50014-induced antinociceptive effect was mediated by endogenous ORs; and (3) Exogenous μ-OR agonist–induced analgesia was enhanced potently by coadministration of an RGS4 inhibitor. These findings demonstrate that spinal RGS4 inhibits endogenous or exogenous OR-mediated antinociceptive effects in the formalin pain test. Our results support coadministration of an RGS4 inhibitor and an OR agonist as a new therapeutic strategy for the management of inflammatory pain. Further study is required to establish the actual functions of RGS4 in the inflammatory pain condition using other inflammatory pain models. ■

DISCLOSURES

Name: Seo-Yeon Yoon, DVM, PhD.
Contribution: This author helped design and conduct the study, analyze and interpret the data, and write the manuscript.
Attestation: Seo-Yeon Yoon has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
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