Protein Kinase A–dependent Spinal α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate–receptor Trafficking Mediates Capsaicin-induced Colon-Urethra Cross-organ Reflex Sensitization

Hsien-Yu Peng, Ph.D.,* Chao-Hsiang Chang, M.D.,† Shin-Jei Tsai, M.D., Ph.D.,‡ Cheng-Yuan Lai, B.S.,§ Kwong-Chung Tung, Ph.D., D.V.M.,‖ Hsi-Chin Wu, M.D., M.Sc.,# Tzer-Bin Lin, Ph.D.**

ABSTRACT

Background: Intracellular redistribution of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPARs) is known to be induced by natural painful stimulation. We tested the hypothesis that that protein kinase A (PKA)-dependent AMPAR trafficking underlies the development of *N*-methyl-D-aspartate receptor–mediated cross-organ sensitization *in vivo*.

Methods: We recorded urethra reflex activity and analyzed immunoblotting of lumbosacral (L6-S2) dorsal horn (DH) tissue obtained from animal preparations after intrathecal 8-bromo-cyclic adenosine monophosphate injection or intracolonic instillation with 8-methyl-*N*-vanillyl-*trans*-6-nonenamide (capsaicin).

Results: Intrathecal 8-bromo-cyclic adenosine monophosphate (300 μM, 10 μl) induced reflex potentiation (81.85 ± 22.21 spikes/stimulation) and increased the number of AMPAR Glu receptor 1 subunits in the membrane fraction of DH (1.8-fold increase *vs.* control). This process was prevented by pretreatment with the PKA inhibitor *N*-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide (10 μM, 10 μl, 2.7 ± 0.8 [mean ± SE] spikes/stimulation) and human thyroid A kinase–anchoring protein (10 μM, 10 μl, 11.5 ± 4.8 spikes/stimulation), an inhibitor of PKA and PKA-A kinase–anchoring protein interactions. Intracolonic capsaicin instillation sensitized the urethra reflex (137.2 ± 62.4 spikes/stimulation) and, relative to control, simultaneously provoked an increase (2.9-fold) in the membrane fraction and a decrease (0.9-fold) in the cytosolic fraction of Glu receptor 1 subunits in DH. Inhibition of PKA activity and disruption of PKA-A kinase–anchoring protein interaction in the DH (2.0 ± 0.6 and 16.7 ± 2.8 spikes/stimulation, respectively) are sufficient to prevent capsaicin-dependent reflex sensitization and AMPAR trafficking in the membrane fraction (0.6- and 0.5-fold increase capsaicin).

Conclusion: Delivery of AMPAR-containing Glu receptor 1 subunits to the membranes of lumbosacral DH neurons through a PKA-dependent pathway contributes to noxious stimulation-induced synaptic strengthening, which plays roles in colon-urethra reflex sensitization.

What We Already Know about This Topic

- Injury or inflammation of one pelvic organ can lead to hypersensitivity and pain in another, but the mechanisms for this cross-sensitization are unknown.

What This Article Tells Us That Is New

- In rats, chemical sensitization of the colon leads to cross-sensitization of the urethra.
- This cross-sensitization requires trafficking of glutamate receptors to the membrane of spinal cord neurons and is dependent on protein kinase A signaling.

◆ This article is accompanied by an Editorial View. Please see: Zuo Z, Sorkin LS: Molecular mechanism of cross-organ reflex sensitization: Should I pay attention to it? ANESTHESIOLOGY 2011; 114:14–5.

* Assistant Professor, Department of Urology, China Medical University Hospital; Assistant Professor, Department of Physiology, School of Medicine, China Medical University, Taichung, Taiwan. † Assistant Professor, # Associate Professor, Department of Urology, China Medical University Hospital, China Medical University. ‡ Associate Professor, Department of Neurology, Chung-Shan Medical University Hospital, Chung-Shan Medical University, Taichung, Taiwan. § Postdoctoral Student, ‖ Professor, Department of Veterinary Medicine, College of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan. ** Professor, Department of Urology, China Medical University Hospital; Professor, Department of Physiology, School of Medicine, China Medical University; Professor, Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, Taiwan.

Received from the Neural Plasticity Research Group, School of Medicine, China Medical University, Taichung, Taiwan. Submitted for publication April 18, 2010. Accepted for publication August 6, 2010. Supported by the National Science Council (Taipei, Taiwan) Grant no. 97-2320-B-040-008-MY3 and NSC-982320-B-040-006-MY3 (to Dr. Lin) and NSC 99-2320-B-039-036 (to Dr. Peng) as well as by the China Medical University (Taichung, Taiwan) CMU99-S-19 and CMU99-106 (to Dr. Lin) and DMR-100–106 (to Dr. Peng). Drs. Wu and Lin contributed equally to this work.

Address correspondence to Dr. Lin: Department of Physiology, School of Medicine, China Medical University, No. 91, Hsueh-Shih Road, Taichung, Taiwan 40201. tblin2@gmail.com. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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ALPHA-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), which are responsible for most excitatory synaptic transmission in the mammalian central nervous system, undergo constant trafficking between the plasma membrane and the cytoplasmic compartment. Alternations of this dynamic process redistribute AMPARs in neurons of the central nervous system and may underlie changes in synaptic efficacy, such as those seen in long-term potentiation (LTP).[1–6] AMPAR trafficking is regulated by a variety of mechanisms. For example, activation of *N*-methyl-D-aspartate (NMDA) receptors, AMPARs, and metabotropic glutamatergic receptors triggers rapid AMPAR internalization.[4] Selective activation of synaptic NMDA receptors has been demonstrated to facilitate AMPAR surface insertion,[7] which plays a key role in dynamics and synaptic accumulation of AMPAR.[4,5]

Although the intracellular cascades leading to AMPAR relocation have been examined recently, the results remain controversial. Studies investigating involvement of different kinases and phosphatases in protein redistribution have suggested that phosphorylation is a major mechanism in regulating intracellular AMPAR trafficking.[8] AMPARs are members of a substrate of protein kinases that include PKA, protein kinase C, and calcium/calmodulin-dependent protein kinase II.[7] Phosphorylation of AMPAR Glu receptor 1 subunit (GluR1) by PKA is closely correlated with redistribution and expression of neural plasticity, which implies that PKA regulates AMPAR trafficking *via* direct phosphorylation of GluR1.[9,10]

Specificity in PKA signaling arises in part from the association of the enzyme with A kinase–anchoring proteins (AKAPs), which target PKA to specific substrates at precise subcellular locations.[11,12] At the postsynaptic density, PKA is tethered to the neuronal anchoring protein AKAP, which, along with its associated enzymes, including PKA, are directed to NMDA receptors or AMPARs.[13] Furthermore, AKAP has been shown to regulate AMPAR phosphorylation and functioning,[14–16] therefore playing an important role in synaptic plasticity.[17]

Evidence suggests that the mechanisms underlying neural plasticity in the brain, including hippocampal LTP, may also apply to the spinal cord.[18–20] Trafficking of AMPAR from the cytosol onto the postsynaptic membrane has been linked to spinal mechanisms of pain modulation, including hyperalgesia and allodynia.[20] We recently reported a glutamatergic NMDA receptor–mediated neural plasticity at the spinal cord level, colon-urethra cross-organ reflex sensitization, which is presumed to underlie viscero-visceral referred pain within the pelvis.[21–23] However, involvement of PKA-dependent AMPAR trafficking in cross-organ sensitization has yet to be established. Therefore, we hypothesized that PKA-dependent AMPAR redistribution underlies the development of NMDA receptor–mediated cross-organ sensitization *in vivo*. Our results demonstrate that PKA-dependent anchoring of PKA—through association with AKAP, which regulates AMPAR surface expression—is crucial to the cross-organ sensitization underlying viscero-visceral referred pain in the lumbosacral spinal cord.

Materials and Methods

Animal Preparations

Four hundred sixty-three female Sprague-Dawley rats (215–320g) were used in this experiment. Study protocols were reviewed and approved by the Institutional Review Board at National Chung Hsing University (Taichung, Taiwan). All efforts were made to minimize the number of animals used throughout this study and animal suffering. Animals were randomized to receive bladder vehicle solution or 8-methyl-*N*-vanillyl-*trans*-6-nonenamide (capsaicin) instillation with/without test agent injections. Surgical procedures for animal preparation were adapted from our previous work.[22] In brief, rats were anesthetized with urethane (1.2 g/kg, intraperitoneally), and a PE-50 intracolonic catheter was inserted into the descending colon (4 cm from the anus) for the dispensation of capsaicin or vehicle solution. A PE-10 catheter was inserted through a slit made in the atlanooccipital membrane and passed caudally to the T13 vertebrae (L6-S2 spinal cord level) for test agent injections. The right pelvic nerve was carefully dissected from the surrounding tissues and transected. Single shocks with a pulse duration of 0.05 ms at a fixed strength that evoked a single action potential in the external urethral sphincter electromyogram were repeated with a frequency of 1 stimulation/30 s (test stimulation [TS]) to stimulate the central stump of the pelvic nerve. The activity of external urethral sphincter electromyogram, which was recorded through a pair of epoxy-coated copper wire electrodes, was amplified 20,000-fold after being filtered (bandpass 30–3,000 Hz) by a preamplifier and was recorded continuously. Twenty rats did not complete the experimental procedures; data from all other animals were included in statistical analysis.

Drug Application

Tested agents administered *via* intrathecal injection were as follows: 4-chloro-homoibotenic acid (CHB; 30, 100, and 300 μM, 10 μl [Tocris Bioscience, Bristol, United Kingdom]), a selective AMPAR GluR1/2 activator; philanthotoxin-74 (PT74; 10 μM, 10 μl [Tocris Bioscience]), a selective AMPAR GluR1/2 antagonist; 8-bromo-cyclic adenosine monophosphate (cAMP; 100, 300, and 1,000 μM, 10 μl [Sigma-Aldrich, St. Louis, MO]), a PKA activator; *N*-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide (H89; 10 μM, 10 μl [Sigma-Aldrich]), a PKA inhibitor; human thyroid AKAP (Ht31; 10 nM, 10 μl [Promega Corporation, Madison, WI]), an inhibitor of the interaction between the RII subunit of PKA (PKARII) and AKAP; MDL-12330A (10 μM, 10 μl; Sigma-Aldrich), an adenylate cyclase inhibitor; NMDA, 10 μM, 10 μl [Sigma-Aldrich]), a selective glutamatergic NMDA receptor agonist; D-2-amino-5-phosphonovalerate (APV; 100 μM, 10 μl [Sigma-Aldrich]), a glutamatergic NMDA receptor antagonist. Alternatively, capsaicin (0.03, 0.1, and 0.3%; Sigma-Aldrich), a natural vanilloid compound, or 0.1 ml lido-

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caine hydrochloride, 0.2% (AstraZeneca Pharmaceuticals, Westminster, United Kingdom), a nerve conduction blocker, was administered intracolonically.

Western Blotting

Thirty minutes after intrathecal application of tested agents and/or 2 h after intracolonic capsaicin instillation, rats were deeply anesthetized, and spinal cords were obtained. The dorsal horn (DH) of the right lumbosacral (L6-S2) spinal cord was dissected for Western blot analysis. In experiments that measured protein expression and GluR1 phosphorylation, total lysate was used for analysis. Alternatively, for GluR1, AKAP, and PKARII expression in membrane and subcellular fractions, we used methods adapted from Galan *et al.*[20] to obtain pure cytosol and crude plasma membrane fraction (P2) from the lumbosacral DH of the same animal. Proteins were incubated in rabbit anti-GluR1 (1:2,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti–Glu receptor 1 subunit (GluR2; 1:2,000; Santa Cruz Biotechnology, Inc.), antiphosphorylated GluR1 (pEphB1/2, 1:2,000; Millipore Corporation, Billerica, MA), rabbit anti-AKAP (1:1,000; Millipore Corporation), and rabbit anti-PKARII (1:1,000; Millipore Corporation). Blots were incubated in peroxidase-conjugated donkey anti-rabbit IgG (1:5,000; Santa Cruz Biotechnology, Inc.) or donkey anti-mouse IgG (1:10,000; Santa Cruz Biotechnology, Inc.). Protein bands were visualized using an enhanced chemiluminescence detection kit (WBKU 0500; Millipore, Billerica, MA) and autoradiography.

RNA Extraction and Quantitative Reverse-Transcription Polymerase Chain Reaction

Total RNA was extracted from dissected right lumbosacral spinal DHs (L6-S2) using RNA extraction kits (74106; Qiagen, Valenica, CA). Reverse transcription was performed using complementary DNA reverse transcription kits (205311; Qiagen Valenica, CA). Complementary DNA samples underwent absolute quantitative real-time polymerase chain reaction on the OneStep apparatus using the default 40-cycle program. TaqMan gene expression assays were purchased from Applied Biosystems (Foster, CA). For each amplicon, polymerase chain reaction efficiency was estimated to be near 1.0 using serial dilutions of complementary DNA. Relative quantities of messenger RNA were estimated using the delta-delta CT method.

Small Interfering RNA of AKAP

The 19-nucleotide duplex and 2-unpaired nucleotide overhangs of the 3′ end were as follows: negative controls, 5′-UUCUCCGAACGUGUCACGUTT-3′ and 3′-TTAAGAGGCUUGCACAGUGCA-5′; AKAP150, 5′-GCAUGUGAUUGGCAUAGAATT-3′ and 3′-TTCGUACACUAACCGUAUCUU-5′.

Using the negative control or small interfering RNA of AKAP solution, we injected 10 uL intrathecally at the T13 vertebrae level of adult rats once daily for 5 days. Animals were allowed 2 days to recover and were then used for reflex activity recording and immunoblotting.

Experimental Protocols

Protocols for assessing effects of electrical stimulation and various kinds of reagents on reflex activity were as follows:

Protocol 1: Baseline Reflex Activity. Single TS shocks (1 stimulation/30 s) were given through stimulation electrodes for 60 min.

Protocol 2. Agonist-induced Reflex Potentiation. After equilibration (usually 30 min), CHB, cAMP, or NMDA was injected *via* intrathecal catheter at 10 min, whereas H89, Ht31, APV, or PT74 was injected 20 min before TS onset to verify drug effects.

Protocol 3. Acute Colon Irritation. Capsaicin (0.1 ml), 0.1%, was instilled into the colon lumen through the catheter. One minute after capsaicin instillation, effects on reflex activity were evaluated by applying TS to the pelvic nerve for 60 min.

Protocol 4. Intracolonic Pharmacologic Testing. A nerve conduction blocker, lidocaine, was instilled *via* intracolonic catheter 10 min before TS to test drug effects on reflex activity.

Protocol 5. Intrathecal Pharmacologic Testing. APV, H89, Ht31, or PT74 was injected *via* the intrathecal catheter 20 min before TS to verify drug effects.

Statistics

The density of specific bands from Western blot analysis was measured using computer-assisted imaging analysis (LAS-300; Fuji, Kanagawa, Japan) and normalized against corresponding loading control bands. Data were analyzed using SigmaPlot (version 10.0; Systat Software, Inc., San Jose, CA). All data are presented as mean ± SE unless otherwise specified. Before performing analysis of variance, the Shapiro-Wilk test was used to ensure normal distribution of data. No statistical power analysis was calculated before the study was conducted. For serial measurements over time (*i.e.*, spikes of external urethra sphincter electromyogram and protein expression in response to capsaicin instillation at different time point), two-way repeated-measures ANOVA was used to assess changes in values before and after treatment. In other cases, one-way ANOVA was used to analyze data. No correction for multiple testing was performed. In all cases, a Tukey test was used to compare means for groups when an adequate F ratio was achieved. Statistical significance was assigned at a P value of less than 0.05.

Results

AMPAR GluR1/2 Subunit Mediates Induction of Reflex Potentiation

We applied vehicle solution or the selective GluR1/2 activator CHB (100 μM, 10 μl, intrathecal) 10 min before TS, which lasted for 60 min. We observed that intrathecal injection of CHB (figs. 1A and B; 81.8 ± 22.2 spikes/stimula-

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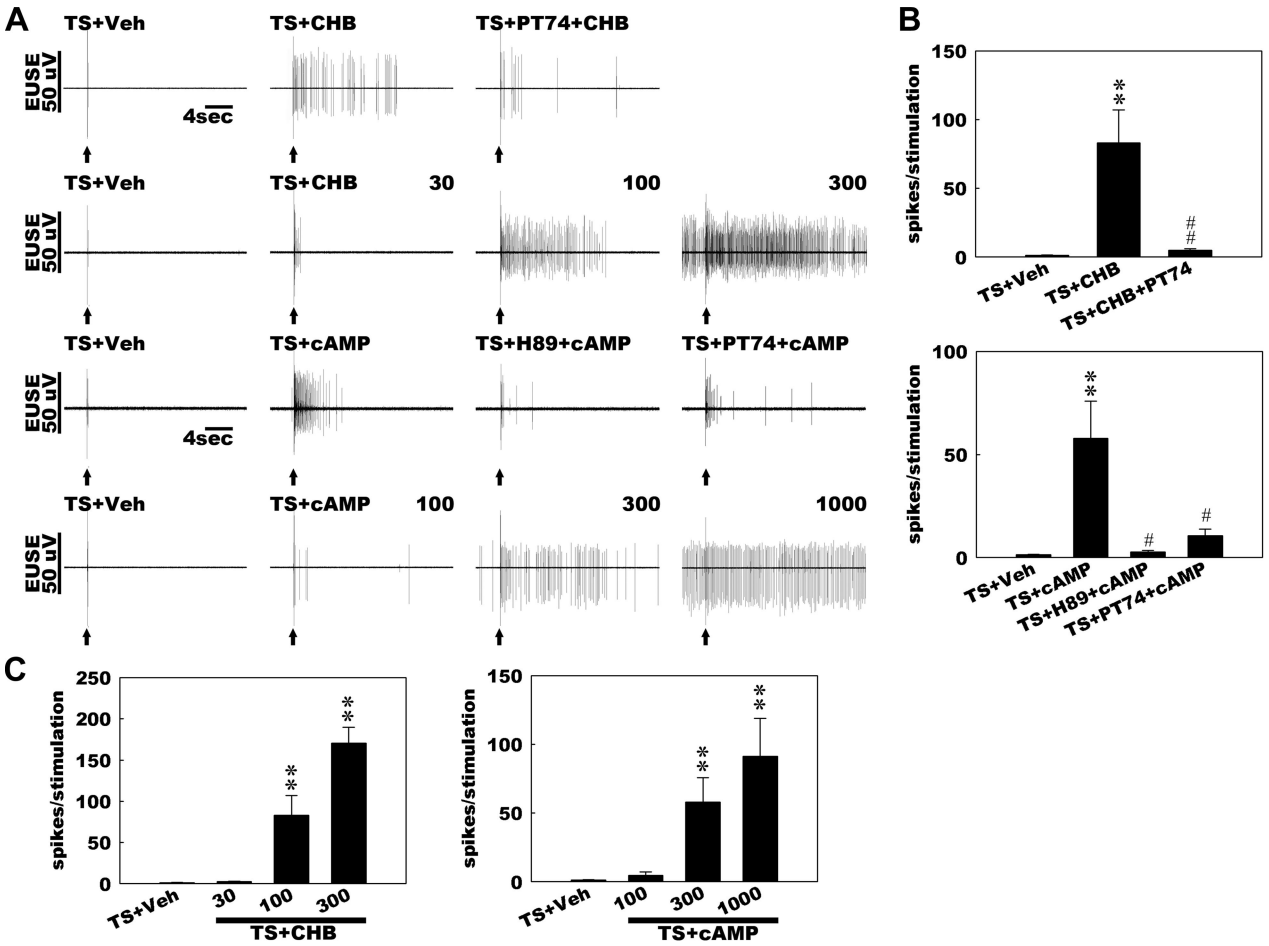

Fig. 1. Protein kinase A–dependent reflex potentiation requires α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors Glu receptor 1 and 2 subunits. (*A*, *B*) Intrathecal 4-chloro-homoibotenic acid (CHB, 100 nM, intrathecal; test stimulation [TS] + CHB, [n = 7]), but not vehicle (Veh) solution injection (n = 7), induced reflex potentiation in the external urethra sphincter electromyogram by increasing mean spike count (** $P = 0.02$). This process was prevented by intrathecal pretreatment with philanthotoxin-74 (PT74, 10 μM, 10 μl; ##$P = 0.04$ *vs.* TS + CHB). Compared with vehicle solution (n = 7), reflex activity of the external urethral sphincter electromyogram was augmented in parallel to tested PT74 concentrations at 30 μM, 100 μM (** $P = 0.002$), and ; sec = seconds; uV = μvolts300 μM ($P = 0.001$). (*A*, *C*) Rather than vehicle solution, intrathecal 8-bromo-cyclic adenosine monophosphate (cAMP, 300 μM, 10 μl) induced reflex potentiation (** $P = 0.008$) that was prevented by intrathecal *N*-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide (H89, 10 μM, 10 μl; ** $P = 0.010$ *vs.* TS + cAMP, n = 7) and PT74 (10 μM, 10 μl; TS + PT74 + cAMP, ** $P = 0.034$). Reflex activity was gradually facilitated when cAMP concentration was increased from 300 nM (** $P = 0.009$ *versus* TS + vehicle, n = 7) to 1,000 nM ($P = 0.007$). EUSE = external urethra sphincter electromyogram.

tion, *$P = 0.02$, n = 7), but not vehicle solution (1.2 ± 0.7 spikes/stimulation, n = 7), induced reflex potentiation characterized by elongated firing in the external urethra sphincter electromyogram. CHB in varying concentrations caused a dose-dependent increase in evoked spike count at 30, 100, and 300 μM, respectively (figs. 1A and C; **$P = 0.002$ in 100 μM, $P = 0.001$ in 300 μM).

Intrathecal pretreatment with PT74 prevented CHB-induced reflex potentiation (5.6 ± 1.7 spikes/stimulation; **$P = 0.004$).

PKA Activation Involvement in Reflex Potentiation Induction

Ten minutes before TS onset, we applied vehicle solution or the PKA activator cAMP (300 μM, 10 μl). When compared with rats that received vehicle solution (n = 7), intrathecal cAMP induced reflex potentiation characterized by elongated firing (48.0 ± 18.2 spikes/stimulation, **$P = 0.008$).

Intrathecal pretreatment with H89 prevented the increase in spike count caused by cAMP (2.7 ± 0.8 spikes/stimulation, #$P = 0.10$ *vs.* TS + cAMP, n = 7). A dose-dependent increase in evoked spike number to the concentration of cAMP was found at 100, 300, and 1,000 μM (**$P = 0.009$ at 300 μM, $P = 0.007$ at 1,000 μM *vs.* TS + vehicle, n = 7).

AMPAR GluR1/2 Subunit Underlies PKA-dependent Reflex Potentiation

Pharmacologic antagonizing of the GluR1/2 subunit by pretreatment with PT74 abolished the cAMP-dependent reflex

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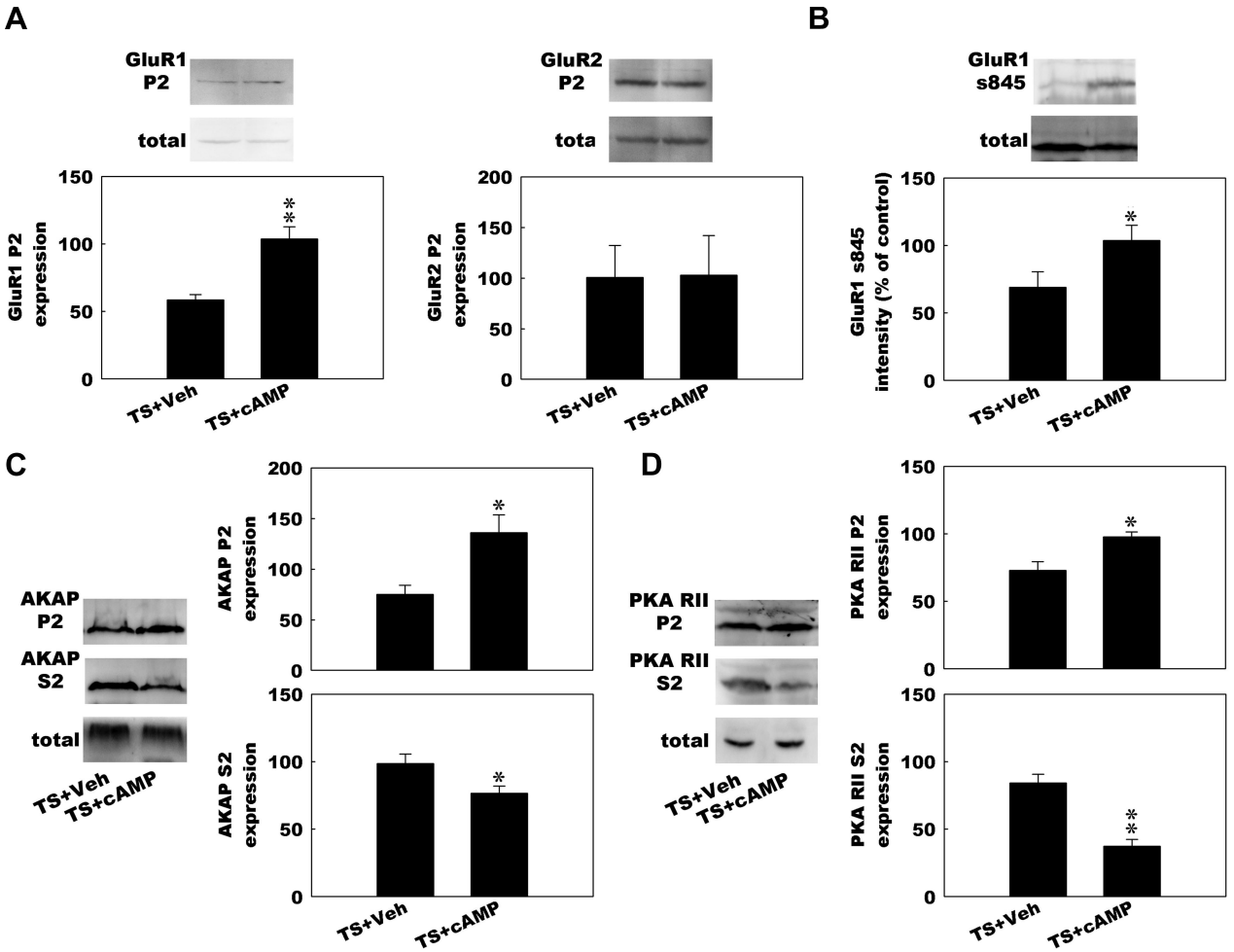

Fig. 2. Phosphorylation of Glu receptor 1 subunit (GluR1) and its residue (pGluR1 s845) with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate–receptor trafficking. (*A*) When compared with the vehicle group (Veh), rats that received intrathecal injection with 8-bromo-cyclic adenosine monophosphate (cAMP, 300 μM, 10 μl; test stimulation [TS] + cAMP) had more GluR1 subunits in the membrane fraction (P2; ** $P = 0.001$ *vs.* TS + vehicle, n = 4) of the lumbosacral (L6–S2) spinal dorsal horn tissue, whereas total expression level (total) was unaffected. However, cAMP had no effect on the intracellular distribution of Glu receptor 1 subunit (GluR2) in dorsal horn tissue ($P = 0.945$, n = 4). (*B*) Rather than total GluR1 protein level, intrathecal cAMP injection enhanced phophorylation levels of GluR1 s845 spinal dorsal horn neurons (* $P = 0.044$) when compared with vehicle solution (n = 4). (*C*, *D*) Likewise, intrathecal cAMP produced an increase in the abundance of A kinase–anchoring proteins (AKAP; * $P = 0.012$) and the RII subunit of protein kinase A (PKA RII; $P = 0.030$) in membrane fractions as well as a corresponding decrease in the pure cytosolic fraction (S2, * $P = 0.0030$ and $P = 0.005$, respectively) when compared with the vehicle group.

potentiation (11.5 ± 4.8 spikes/stimulation, *$P = 0.034$ *vs.* TS + cAMP, n = 7).

PKA Activation Increases GluR1 Surface Expression in DH Neurons

Subcellular fractioning of proteins from rat lumbosacral (L6-S2) DH neurons were conducted, as was immunoblotting with antibodies against GluR1/2 subunits.[20] When compared with the vehicle group (fig. 2A; n = 4), rats that received intrathecal cAMP injection had a 1.8-fold increase in GluR1 subunit abundance in membrane fraction whereas total expression level of GluR1 was unaffected (*$P = 0.01$). In contrast to the pronounced effects on GluR1, cAMP did not alter intracellular distribution of GluR2 in spinal DH tissue ($P = 0.945$ *vs.* TS + vehicle, n = 4).

PKA Activation Increases GluR1 Surface Expression in Association with GluR1S845 Phosphorylation

We conducted immunoblotting with antibodies specifically against the phosphorylated serine 845 residue in the AMPAR GluR1 subunit (GluR1S845) and against the subunit itself (pGluR1). We observed that, rather than total GluR1 protein level (fig. 2B), intrathecal cAMP injection enhanced phophorylation at GluR1S845 by 1.4-fold in the total lysate of DH neurons when compared with vehicle solution treatment (*$P = 0.044$, n = 4).

PKA Regulates Anchoring of PKA to AKAP and Their Intracellular Distribution

Next, subcellular fractions of lumbosacral (L6-S2) spinal DH tissue obtained from rats that received vehicle solution

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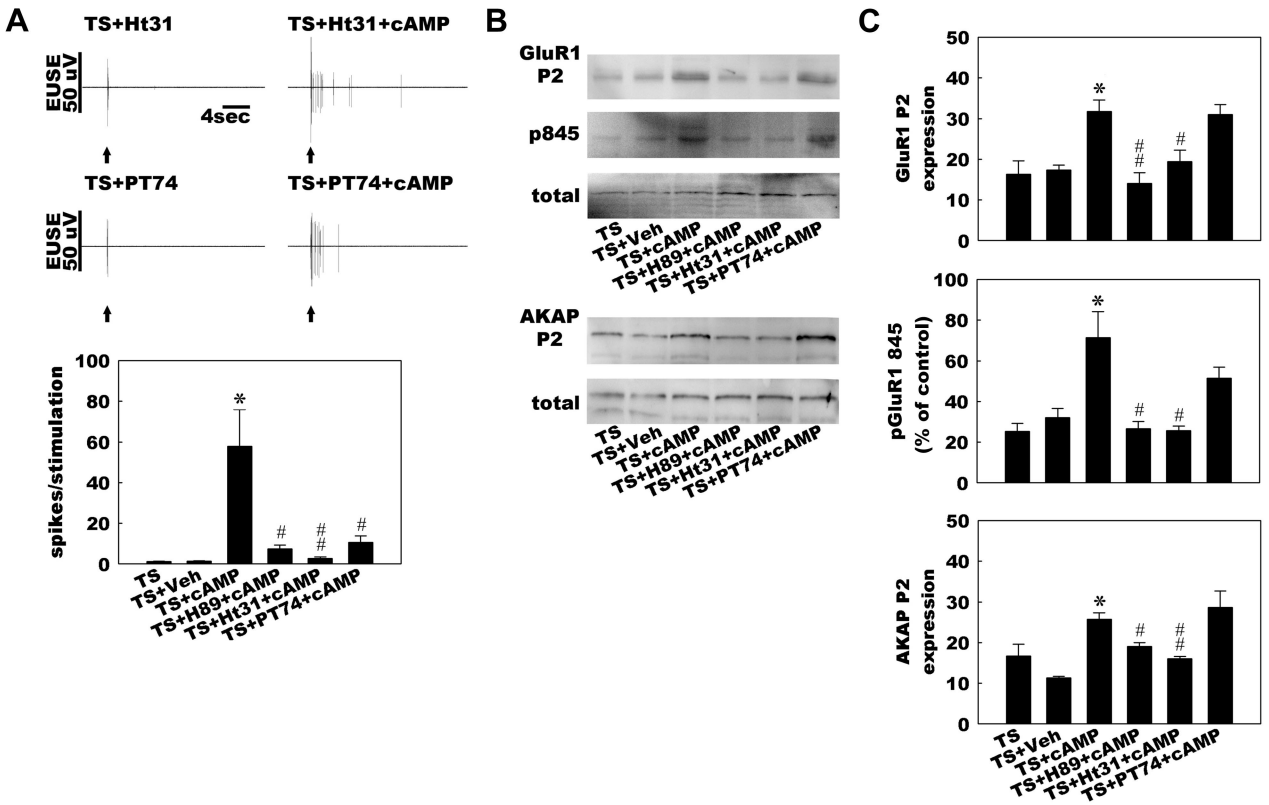

Fig. 3. Anchorage of A kinase–anchoring proteins (AKAP) to protein kinase A and activation of the Glu receptor 1 subunits (GluR1) mediates protein kinase A–dependent reflex potentiation. (*A*) Intrathecal 8-bromo-cyclic adenosine monophosphate (cAMP; 300 μM, 10 μl; test stimulation [TS] + cAMP), but not vehicle solution (Veh; n = 7), induced reflex potentiation in external urethra sphincter electromyogram activity by significantly increasing mean spike count evoked by TS (* P = 0.019). This effect was abolished by pretreatment with intrathecal *N*-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide (H89; # P = 0.024), human thyroid A kinase–anchoring protein (Ht31; # P = 0.001), and philanthotoxin-74 (PT74, 10 μM, 10 μl; P = 0.034). (*B*, *C*) Compared with vehicle solution (n = 4), intrathecal cAMP up-regulated the expression of phosphorylated Glu receptor 1 subunit serine 845 residue (pGluR1 s845) in total lysate (* P = 0.036) as well as GluR1 (* P = 0.016) and AKAP (* P = 0.032) in the membrane fraction (P2) of lumbosacral (L6–S2) dorsal horn neurons. This process was reversed by pretreatment with H89 (## P = 0.007 in GluR1, # P = 0.046 in pGluR1, # P = 0.020 in AKAP *vs.* TS + cAMP, n = 4), Ht31 (## P = 0.018 in GluR1, ## P = 0.036 in pGluR1, ## P = 0.004 in AKAP), but not PT74 (P = 0.845 in GluR1, P = 0.231 in pGluR1, P = 0.483 in AKAP). EUSE = external urethra sphincter electromyogram; sec = seconds; uV = μvolts.

or cAMP injections were immunoblotted using antibodies against AKAP and PKARII. Intrathecal cAMP (figs. 2C and D) produced a pronounced increase in the abundance of AKAP and PKARII, by 1.9- and 1.3-fold, respectively, in the membrane fraction and a corresponding decrease of 0.8- and 0.5-fold in the pure cytosolic fraction (*P = 0.012 and P = 0.030, respectively, n = 4) when compared with the vehicle group (*P = 0.030, **P = 0.005, respectively, n = 4).

Involvement of PKA Anchorage to AKAP in Reflex Potentiation

When compared with animals that received intrathecal cAMP (fig. 3A; 58.0 ± 16.7 spikes/stimulation, *P = 0.019 *vs.* TS + vehicle, n = 7), pretreatment with Ht31 abolished PKA-dependent reflex potentiation (4.2 ± 1.5 spikes/stimulation, ##P = 0.001 *vs.* TS + cAMP, n = 7).

In line with electrophysiologic recordings, immunoblotting illustrations showed that the increment of pGluR1 in total lysate (figs. 3B and C; GluR1S845, 2.0-fold, *P = 0.036 *vs.* TS + vehicle, n = 4) as well as GluR1 (1.7-fold, *P = 0.016) and AKAP (2.8-fold, *P = 0.032) in the membrane fraction of spinal DH neurons caused by intrathecal cAMP were all reversed by Ht31 pretreatment (#P = 0.018 in GluR1, #P = 0.036 in pGluR1, ##P = 0.004 in AKAP *vs.* TS + cAMP, n = 4), but not by PT74 (P = 0.845 in GluR1, P = 0.231 in pGluR1, P = 0.483 in AKAP).

Capsaicin-induced Cross-organ Sensitization and AMPAR Distribution

We instilled vehicle solution or 0.1 ml capsaicin, 0.1%, into the lumen of the descending colon and then evoked urethra reflex activity. We found that intracolonic instillation of vehicle solution did not affect the baseline reflex activity evoked by TS (fig. 4A). Although capsaicin induced no spontaneous background discharge in electromyogram activity, it dramatically increased the spike count evoked by TS at 1 min

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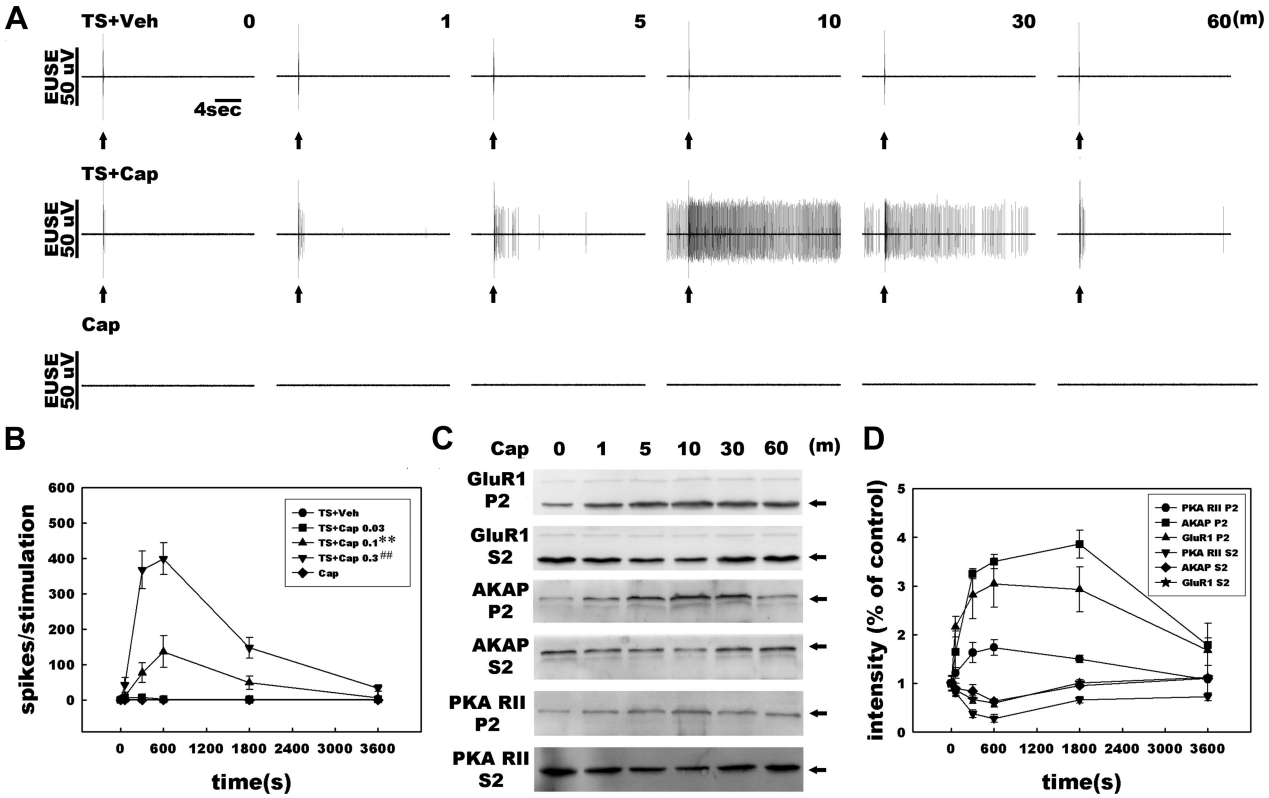

Fig. 4. Intracolonic 8-methyl-*N*-vanillyl-*trans*-6-nonenamide (0.1 ml capsaicin, 0.1% [Cap]) instillation induces cross-organ sensitization in association with distribution of α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate receptors. (*A*, *B*) Intracolonic instillation of vehicle solution (Veh) did not affect the reflex activity evoked by the test stimulation (TS). Although capsaicin induced no spontaneous background discharge in external urethra sphincter electromyogram activity, it dramatically increased the spike number evoked by the stimulation in a dose-dependent manner when drug concentration was increased up to 0.3%. ** $P < 0.01$ compared with TS+Veh; ## $P < 0.01$ compared with TS+Veh. (*C*, *D*) Immunoblotting showed that, when compared with preinstillation protein levels, capsaicin caused rapid increases in the expression of the Glu receptor 1 subunit (GluR1), PKA-A kinase–anchoring protein (AKAP), and the RII subunit of protein kinase A (PKA RII) in membrane fractions (P2) as associated with corresponding decreases in cytosolic fraction (S2) at 10–30 min postinstillation. EUSE = external urethra sphincter electromyogram; sec = seconds; uV = μvolts.

postinstillation, peaking at 5–10 min postinstillation. Capsaicin at varying concentrations caused a dose-dependent increment in evoked spike counts ranging up to 0.3% (fig. 4B; n = 7). Next, we performed subcellular fractioning of proteins obtained from lumbosacral DH neurons at different time intervals after intracolonic capsaicin instillation.

Immunoblotting with specific antibodies against GluR1, AKAP, and PKARII was then used. Western blots showed that, when compared with protein levels before instillation, capsaicin caused a rapid increase in GluR1, AKAP, and PKARII expression in the membrane fraction (figs. 4C and D) with a corresponding decrease in the pure cytosolic fraction (n = 7).

Capsaicin-induced Reflex Sensitization Involves PKA-dependent AMPAR Distribution Mediated by PKA-AKAP Assembly

When compared with animals that received capsaicin instillation (137.2 ± 62.4 spikes/stimulation, $*P = 0.025$ *vs.* TS + vehicle, n = 7), H89 prevented capsaicin-induced reflex sensitization (figs. 5A and B; 2.0 ± 0.6 spikes/stimulation, $*P = 0.019$ *vs.* TS + capsaicin, n = 7) and reduced expression of total lysate for pGluR1 (0.6-fold, $\#\#P = 0.005$) as well as membrane fractions of GluR1 and AKAP (figs. 5C and D; 0.6- and 0.3-fold, respectively, $\#\#P < 0.001$ each). In addition, Ht31 prevented both reflex sensitization (6.7 ± 2.8 spikes/stimulation, $*P = 0.028$) as well as up-regulation of pGluR1 (0.3-fold, $\#\#P < 0.001$) as well as membrane fraction of GluR1 and AKAP (0.5- and 0.3-fold, respectively, $\#\#P < 0.001$ each) caused by capsaicin.

In PT74 pretreated animal preparations, we found that the development of reflex sensitization was dramatically antagonized (12.1 ± 3.0 spikes/stimulation, $\#P = 0.024$ *vs.* TS + capsaicin, n = 7). However, the increment of pGluR1 in total lysate ($P = 0.564$) and membrane fractions of GluR1 and AKAP ($P = 0.074$ and $P = 0.065$, respectively) caused by intracolonic capsaicin instillation were not affected by PT74 pretreatment.

Colonic Afferent Fibers Mediate PKA-dependent Reflex Sensitization

We then injected 0.1 ml lidocaine, 2%, into the lumen of the descending colon before capsaicin instillation. Intracolonic

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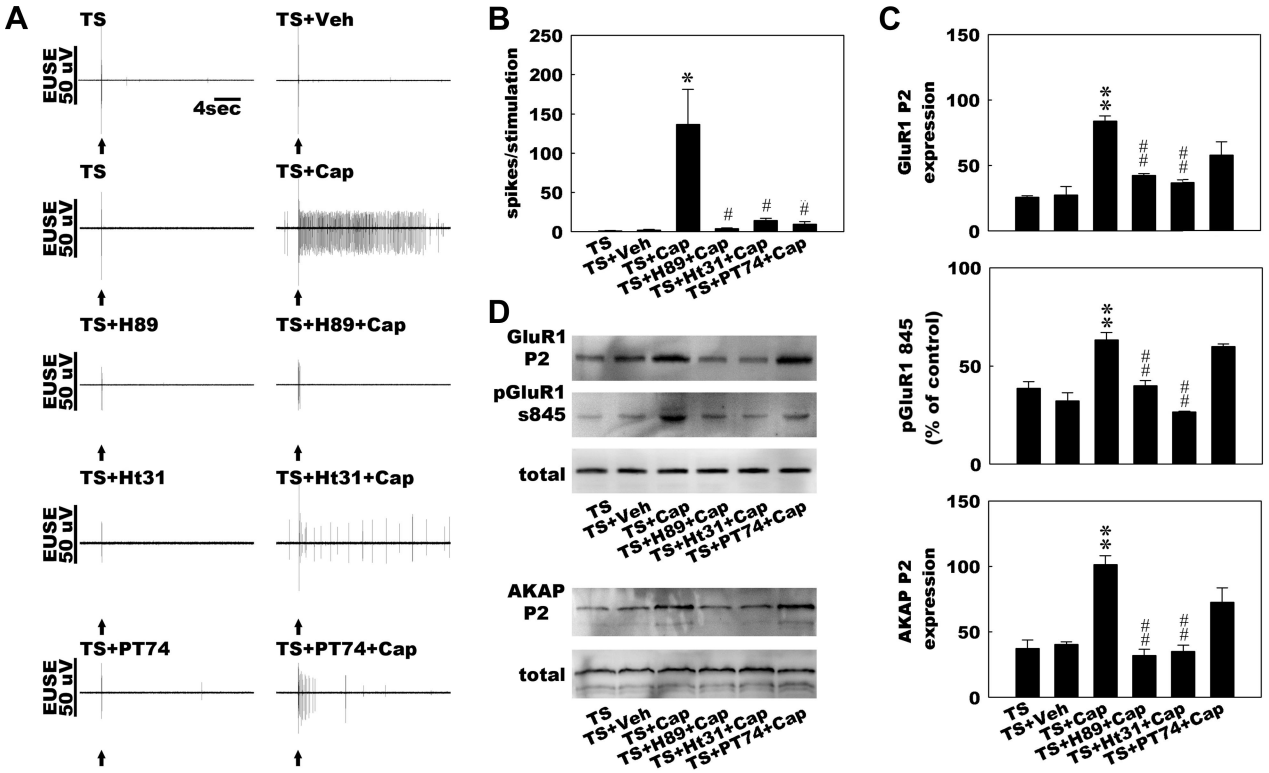

Fig. 5. Reflex sensitization induced by intracolonic 8-methyl-*N*-vanillyl-*trans*-6-nonenamide (0.1 ml capsaicin, 0.1% [Cap]) instillation involves protein kinase A–dependent distribution of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors mediated by protein kinase A and the A kinase–anchoring protein (AKAP) assembly. (*A*, *B*) Intracolonic instillation of capsaicin, but not vehicle solution (Veh; n = 7), sensitized reflex activity in the external urethra sphincter electromyogram by increasing mean spike count (137.2 ± 62.4 spikes/stimulation, * P = 0.025). This process was prevented by intrathecal *N*-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide (H89, 10 μM, 10 μl; 2.0 ± 0.6 spikes/stimulation, # P = 0.019 *vs.* test stimulation [TS] + capsaicin, n = 7), human thyroid A kinase–anchoring protein (Ht31, 10 μM, 10 μl; 16.7 ± 2.8 spikes/stimulation, P = 0.028) and philanthotoxin-74 (PT74, 10 μM, 10 μl; 42.1 ± 3.0 spikes/stimulation, P = 0.024). (*C*, *D*) Immunoblotting shows that, when compared with protein levels in animals that received vehicle solution (n = 4), capsaicin caused a rapid increase in the phosphorylated Glu receptor 1 subunit serine 845 residue (pGluR1 s845) in total lysate (** P = 0.04) as well as GluR1 and AKAP expression in membrane fractions (P2; both ** $P < 0.001$). This process was reversed by H89 (## $P < 0.001$ in GluR1, ## P = 0.005 in pGluR1, ## $P < 0.001$ in AKAP *vs.* TS + 8-bromo-cyclic adenosine monophosphate [cAMP], n = 4) and Ht31 (all ## $P < 0.001$ in GluR1, pGluR1, and AKAP), but not by PT74 (P = 0.074 in GluR1, P = 0.564 in pGluR1, P = 0.065 in AKAP). EUSE = external urethra sphincter electromyogram; sec = seconds; uV = μvolts.

instillation with capsaicin (fig. 6A; 139.1 ± 41.8 spikes/stimulation, *P = 0.012 *vs.* TS + vehicle, n = 7), but not vehicle solution, induced reflex sensitization characterized by an elongated reflex activity.

Intracolonic lidocaine pretreatment did not affect TS-evoked baseline reflex activity, although it reversed the capsaicin-induced reflex sensitization (2.1 ± 0.2 spikes/stimulation, #P = 0.017 *vs.* TS + capsaicin, n = 7). Consistent with electrophysiologic recordings, when compared with vehicle solution (figs. 6B and C; n = 4), capsaicin instillation up-regulated pGluR1 in total lysate (2.2-fold, *P = 0.042) as well as GluR1 and AKAP in membrane fractions (1.7-fold, **P = 0.007 in GluR1; 2.1-fold, *P = 0.012 in AKAP *vs.* TS + vehicle, n = 4), all of which was prevented by intracolonic lidocaine pretreatment (0.7-fold, ##P = 0.001 in GluR1; 0.3-fold, #P = 0.017 in pGluR1; 0.4-fold, ##P = 0.005 in AKAP *vs.* TS + capsaicin, n = 4).

Immunoblotting illustrations from coprecipitations, obtained from lumbosacral (L6-S2) DH neurons in animals that received intracolonic capsaicin instillation demonstrated that AKAP, PKARII, and AMPAR GluR1 subunits (fig. 6D) were enriched in the AKAP 79 immunocomplex. However, synapse-associated protein 97, a membrane-associated guanylate kinase family protein, did not appear in the immunocomplex. In addition, up-regulation of AKAP, PKARII, and GluR1 in the immunocomplex were all antagonized by intracolonic pretreatment with lidocaine.

PKA-dependent AMPAR Surface Insertion Mediates NMDA Receptor-elicited Reflex Potentiation

Electromyogram recording demonstrated that, when compared with vehicle solution (n = 7), NMDA-induced reflex potentiation in a manner characterized by elongated firing (figs. 7A and B; 24.1 ± 4.8 spikes/stimulation, $P < 0.001$). This process was pre-

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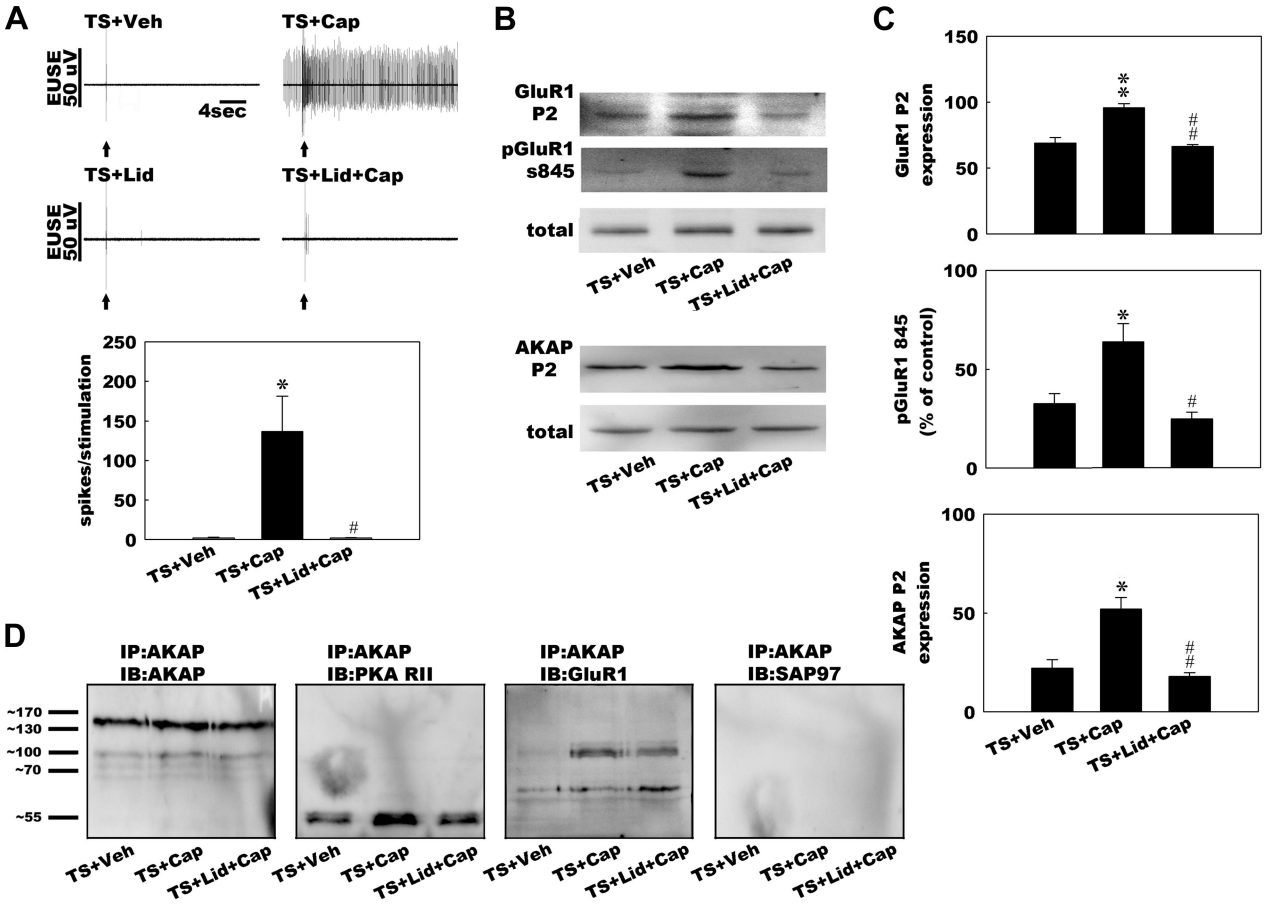

Fig. 6. Afferent fibers arising from the descending colon participate in reflex sensitization induced by 8-methyl-*N*-vanillyl-*trans*-6-nonenamide (0.1 ml capsaicin, 0.1% [Cap]) and mediated by PKA-AKAP–dependent AMPAR trafficking. (*A*) Intracolonic capsaicin (* P = 0.012 *vs.* test stimulation [TS] + vehicle [Veh], n = 7) but not vehicle solution instillation induced reflex sensitization in the external urethra sphincter electromyogram. This process was prevented by pretreatment with 0.1 ml lidocaine hydrochloride, 0.2% (Lid; # P = 0.017 *vs.* TS + capsaicin, n = 7). (*B*, *C*) Representative immunoblotting and summarized data showed that, when compared with protein levels in animals that received vehicle solution (n = 4), capsaicin caused a rapid increase in the phosphorylated Glu receptor 1 subunit serine 845 residue (pGluR1 s845) in the total lysate (* P = 0.042) as well as membrane fractions (P2) of GluR1 and AKAP expressions (** P = 0.007 and P = 0.012, respectively, *vs.* TS + vehicle, n = 4). This process was prevented by lidocaine (## P = 0.001 in GluR1, # P = 0.017 in pGluR1, ## P = 0.005 in AKAP *vs.* TS + capsaicin, n = 4). (*D*) Immunoblotting, obtained from lumbosacral L6–S2 dorsal horn neurons of animals that received intracolonic capsaicin instillation, demonstrated that AKAP, PKA RII, and AMPAR GluR1 subunits (PKA RII, AKAP, and GluR1, respectively) but not synapse-associated protein 97 (SAP97) were enriched in the AKAP 79 immunocomplex. Up-regulation of PKA RII, AKAP, and GluR1 in the immunocomplex were all reversed by lidocaine pretreatment. AKAP = PKA-A kinase–anchoring protein; AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors; EUSE = external urethra sphincter electromyogram; PKA = protein kinase A; PKA RA II = RII subunit of protein kinase A; sec = seconds; uV = μvolts.

vented by pretreatment with APV (2.8 ± 0.4 spikes/stimulation, ## P < 0.001 *vs.* TS + NMDA, n = 7).

Immunoblotting of lumbosacral DH neurons (L6-S2) showed that, when compared with vehicle solution (figs. 7C and D; n = 4), intrathecal NMDA induced an agonist-elicited up-regulation of pGluR1 in total lysate (3.6-fold, * P = 0.019) as well as GluR1 and AKAP in the membrane fractions (3.4-fold, ** P = 0.003 in GluR1; 1.6-fold, * P = 0.034 in AKAP) that was prevented by APV pretreatment (## P = 0.004 in GluR1, # P = 0.017 in pGluR1, # P = 0.027 in AKAP *vs.* TS + NMDA, n = 4). Moreover, agonist-elicited reflex potentiation (17.5 ± 1.9 spikes/stimulation, ## P < 0.001) and increments of pGluR1 in total lysate (0.4-fold, # P = 0.030), as well as GluR1 (0.5-fold, # P = 0.026) and AKAP (0.4-fold, P = 0.042) in membrane fractions, were all prevented by pretreatment with MDL-12330A.

Reflex Sensitization Involves NMDA Receptor–mediated PKA-dependent AMPAR Trafficking

We then administered APV or MDL-12330A 10 min before intracolonic capsaicin instillation. Electromyography demonstrated that capsaicin-dependent reflex sensitization was antagonized by pretreatment with APV (figs. 8A and B; 6.2 ± 2.2 spikes/stimulation, # P = 0.019 *vs.* TS + capsaicin, n = 7) and MDL-12330A (11.8 ± 4.1 spikes/stimulation, # P = 0.022).

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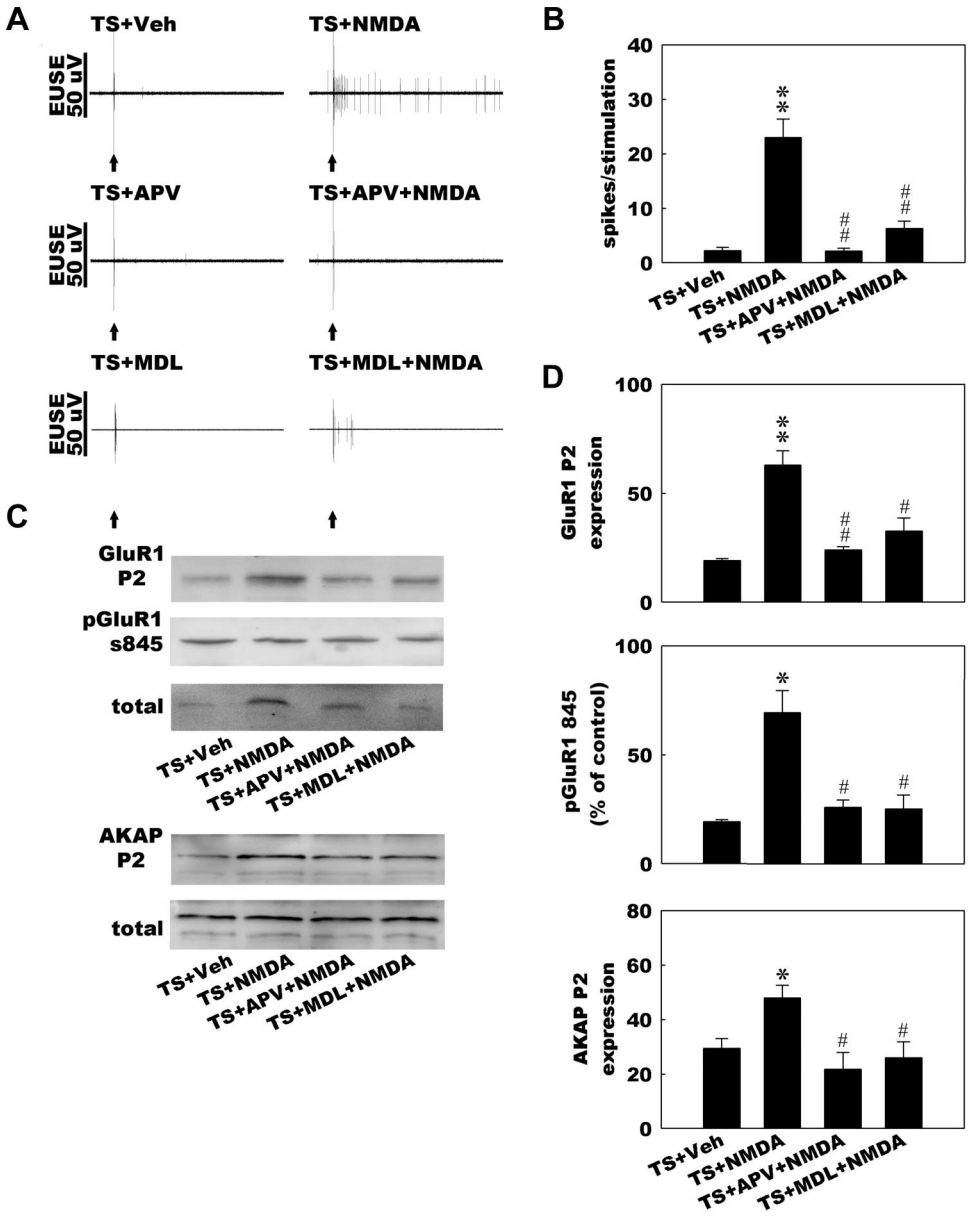

Fig. 7. PKA-dependent AMPAR surface insertion mediates *N*-methyl-D-aspartate (NMDA) receptor–elicited spinal reflex potentiation. (*A*, *B*) When compared with vehicle solution (Veh, n = 7), intrathecal NMDA (10 μM, 10 μl; n = 7) induced reflex potentiation in external urethra sphincter electromyogram activity by increasing spike count (24.1 ± 4.8 spikes/stimulation, ** $P < 0.001$). This process was prevented by pretreatment with intrathecal D-2-amino-5-phosphonovalerate (10 μM, 10 μl [APV]; 2.8 ± 0.4 spikes/stimulation, ## $P < 0.001$) and MDL-12330A (10 μM, 10 μl [MDL]; 7.5 ± 1.8 spikes/stimulation, ## $P < 0.001$). (*C*, *D*) Immunoblotting showed that, when compared with protein levels in animals that received vehicle solution (n = 4), intrathecal NMDA caused rapid increase in phosphorylated Glu receptor 1 subunit serine 845 residue (pGluR1S845) in total lysate (* $P = 0.019$) as well as GluR1 (** $P = 0.003$) and AKAP (* $P = 0.034$) expression in membrane fractions (P2). This process was prevented by APV (## $P = 0.004$ in GluR1, # $P = 0.017$ in pGluR1, # $P = 0.027$ in AKAP *vs.* TS + NMDA, n = 4) and MDL-12330A (# $P = 0.026$ in GluR1, $P = 0.020$ in pGluR1, $P = 0.042$ in AKAP). AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors; AKAP = PKA-A kinase–anchoring protein; EUSE = external urethra sphincter electromyogram; PKA = protein kinase A; sec = seconds; test stimulation = TS; uV = μvolts.

Immunoblotting of lumbosacral DH neurons (L6-S2) showed correlated increases of pGluR1 in total lysate (2.0-fold, ** $P < 0.001$ *vs.* TS + capsaicin, n = 4) as well as GluR1 (2.0-fold, ** $P < 0.001$) and AKAP (1.6-fold, * $P = 0.042$) in membrane fractions (figs. 8C and D) were caused by capsaicin instillation, when compared with vehicle solution (n = 4).

Pretreatment with APV (0.4-fold, ## $P = 0.004$ in GluR1; 0.5-fold, # $P = 0.031$ in pGluR1; 0.5-fold, # $P = 0.034$ in AKAP *vs.* TS + capsaicin, all n = 4) and MDL-12330A (0.4-fold, # $P = 0.015$ in GluR1; 0.6-fold, # $P = 0.023$ in pGluR1; 0.5-fold, # $P = 0.048$ in AKAP *vs.* TS + capsaicin, all n = 4) both prevented capsaicin-dependent expression of pGluR1 as well as membrane

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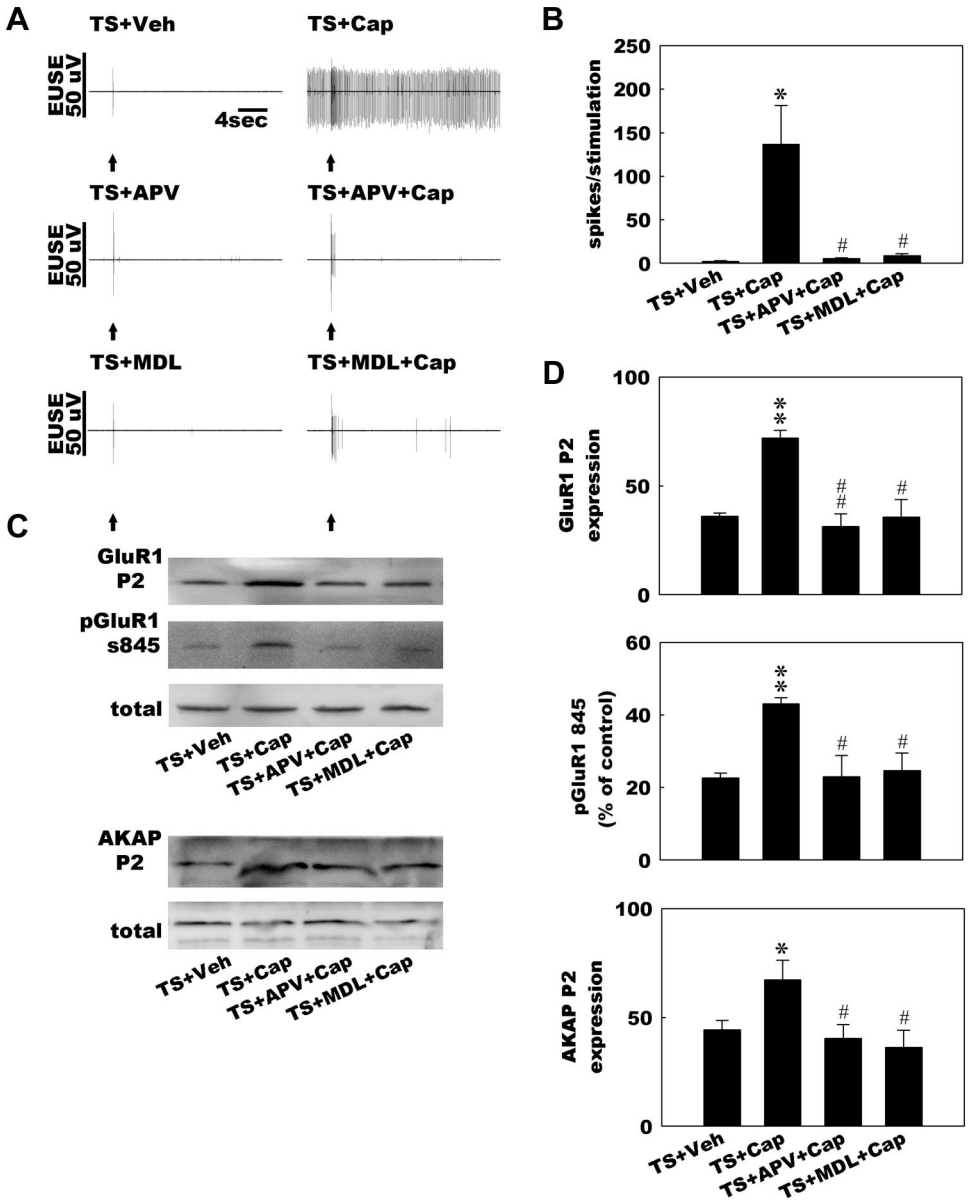

Fig. 8. PKA-dependent AMPAR surface insertion mediates reflex sensitization elicited by 8-methyl-*N*-vanillyl-*trans*-6-nonenamide (0.1 ml capsaicin, 0.1% [Cap]). (*A*, *B*) When compared with vehicle solution (n = 7), intracolonic capsaicin instillation induced reflex sensitization in external urethra sphincter electromyogram activity by increasing mean spike count (136.5 ± 48.2 spikes/stimulation, **P* = 0.012). This process was prevented by pretreatment with intrathecal D-2-amino-5-phosphonovalerate (10 μM, 10 μl [APV]; 6.2 ± 2.2 spikes/stimulation, # *P* = 0.019 *versus* TS + capsaicin, n = 7) and MDL-12330A (10 μM, 10 μl [MDL]; 11.8 ± 4.1 spikes/stimulation, # *P* = 0.022). (*C*, *D*) Immunoblotting showed that, when compared with protein levels in animals that received vehicle solution (n = 4), intracolonic capsaicin instillation caused expression of the phosphorylated Glu receptor 1 subunit serine 845 residue (pGluR1 s845) in total lysate (** *P* < 0.001) as well as membrane fractions (P2) of GluR1 (** *P* < 0.01) and AKAP (* *P* = 0.042. This process was prevented by APV (## *P* = 0.004 in GluR1, # *P* = 0.031 in pGluR1, # *P* = 0.034 in AKAP *vs.* TS + capsaicin, n = 4) and MDL-12330A (# *P* = 0.015 in GluR1, *P* = 0.023 in pGluR1, and *P* = 0.048 in AKAP). AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors; AKAP = PKA-A kinase–anchoring protein; EUSE = external urethra sphincter electromyogram; PKA = protein kinase A; sec = seconds; TS = test stimulation; uV = μvolts.

fraction of GluR1 as well as membrane fraction of GluR1 and AKAP.

PKA-dependent Cross-organ Sensitization Involves AKAP

We next sought to suppress AKAP expression using specific small interfering RNA (siAKAP). AKAP transcription was detected using real-time reverse transcription polymerase chain reaction, which matched the expression size on agarose gel (fig. 9A). Comparison of AKAP messenger RNA expression by real-time reverse transcription polymerase chain reaction showed a 0.5-fold decrease in AKAP expression for the siAKAP group when compared with control animals (**P* = 0.015, n = 4).

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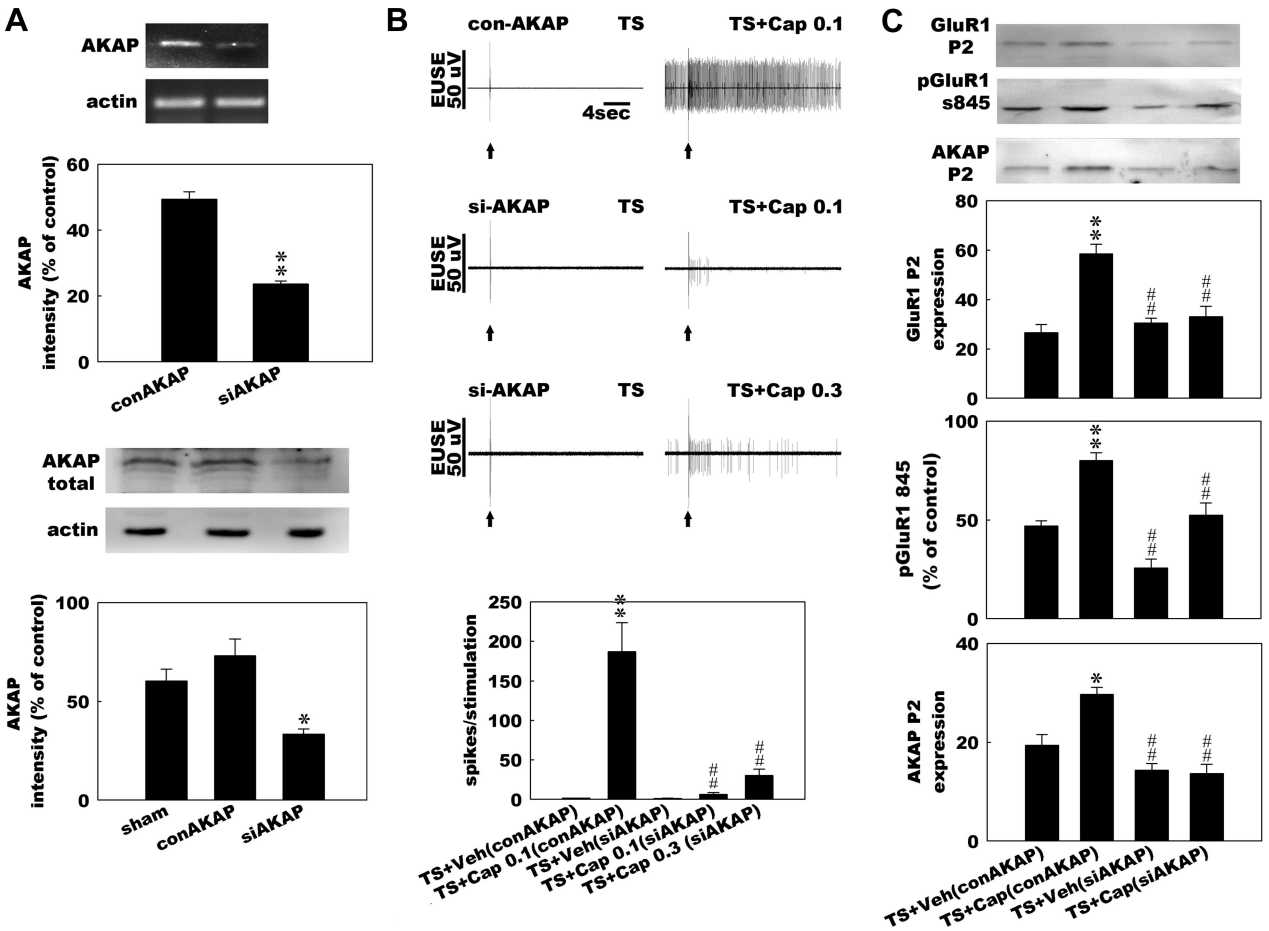

Fig. 9. AKAP mediates PKA-dependent cross-organ sensitization of the urethra reflex. (*A*) Total RNA extracted form the lumbosacral dorsal horn tissue obtained from control (conAKAP) and siAKAP animals, as well as transcription of gene encoding AKAP, were assessed using real-time reverse transcription polymerase chain reaction with AKAP-specific cloning primers. Expression of AKAP messenger RNA in siAKAP rats was significantly lower than that in control animals (** $P < 0.001$, n = 4). Immunoblotting demonstrated that, when compared with controls, small interfering RNA directly knocked-down the expression level of AKAP (* $P = 0.015$, n = 7). (*B*) Reflex sensitization caused by intracolonic 8-methyl-*N*-vanillyl-*trans*-6-nonenamide (0.1 ml capsaicin, 0.1% [Cap]; 192.3 ± 42.1 spikes/stimulation, ** $P < 0.001$ *versus* test stimulation [TS] + vehicle in controls) in siAKAP animals was remarkably attenuated (# $P < 0.001$, n = 7) even though capsaicin concentration was increased to 0.3%. (*C*) Correlated up-regulation of phosphorylated Glu receptor 1 subunit serine 845 residue (pGluR1 s845) in total lysate (** $P = 0.002$ *vs.* TS + vehicle, n = 4) as well as GluR1 and AKAP in the membrane fractions (P2; n = 4) caused by capsaicin instillation was remarkably attenuated in the siAKAP group when compared with controls (## $P = 0.004$ in GluR1, $P = 0.020$ in pGluR1 and ## $P = 0.003$ in AKAP *vs.* TS + vehicle, n = 4). AKAP = PKA-A kinase–anchoring protein; EUSE = external urethra sphincter electromyogram; PKA = protein kinase A; sec = seconds; siAKAP = suppressed AKAP expression using specific small interfering RNA; uV = μvolts.

Immunoblotting demonstrated that, when compared with controls (n = 7), small interfering RNA directly knocked down expression of AKAP (*$P < 0.05$). Reflex sensitization caused by intracolonic capsaicin (fig. 9B; 192.3 ± 42.1 spikes/stimulation, $P < 0.001$) in siAKAP animals (n = 4) was remarkably attenuated when compared with controls, even though capsaicin concentration was increased. The correlated up-regulation of the pGluR1 in total lysate (0.6 fold, ##$P = 0.020$), as well as GluR1 (0.5-fold, ##$P = 0.004$) and AKAP (0.4 fold, ##$P = 0.003$) in the membrane fractions (fig. 9C), caused by capsaicin instillation, was remarkably attenuated in the siAKAP group when compared with controls.

Discussion

In this study, we found that activation of PKA, which subsequently anchors PKA to AKAP, induced GluR1S845 phosphorylation in association with AMPAR delivery from cytosol to plasma membranes. Moreover, such PKA-elicited AMPAR trafficking is involved in NMDA receptor–mediated cross-organ urethra reflex sensitization caused by acute intracolonic capsaicin instillation.

The cross-innervations of visceral organs in the pelvic cavity offer a complex sensory pathway within the spinal cord that is presumed to be essential for physiologic regulation and integration of sexual, bowel, and bladder func-

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tions.[24–26] Moreover, such complicated communication in the nervous system may also underlie the pathophysiologic mechanisms of viscero-visceral referred pain, which is manifested as injury or inflammation in one pelvic organ leading to modifications in others.[27–30] By instilling capsaicin into the descending colon, we demonstrated that activation of nociceptive afferent fibers in the descending colon sensitized urethra reflex activity in a cross-organ manner. This cross-organ reflex sensitization might, at least in part, mimic the pathophysiologic conditions that occur during acute colon irritation. This study could provide an animal model not only for pathophysiologic mechanisms underlying the high concurrence of urologic, gynecologic, and gastrointestinal pain in the pelvis, but also for the development of effective pharmacologic strategies for pelvic pain treatment.

AMPAR GluR1 subunits have been detected in spinal cord locations involved in nociceptive processing, most notably the small neurons of lamina I and II.[31,32] The association with unmyelinated primary afferent markers, including calcitonin gene-related peptide and B4 isolectin, demonstrates that, rather than in synapses made by interneurons, GluR1-containing AMPAR is preferentially located in primary afferent synapses.[33] *In vivo* studies that investigate pain modulation have proposed a spinal mechanism where strengthening of the link between primary afferents and GluR1-containing interneurons could lead to hyperalgesia.[20,34] Previous investigation of AMPAR trafficking in the spinal cord has found that, in animal preparations with intact central nervous systems, intracolonic capsaicin instillation provokes the appearance of AMPAR-mediated nociceptive responses associated with recruitment of GluR1, but not GluR2/3, subunits to synapses.[20] Our present results show that both pharmacologic activation of PKA by intrathecal cAMP injection and acute colon irritation caused by capsaicin instillation induce delivery of GluR1, but not GluR2, from the cytosol to the plasma membranes of lumbosacral DH neurons. These results are consistent with the proposal that GluR1 insertion into the plasma membrane is an inducible and tightly regulated process wherein GluR2/3 subunits are constitutively cycled in and out of the plasma membrane to maintain typical transmission.[2,35] Moreover, our results suggest that AMPAR trafficking at the spinal cord level is also crucial for the development of cross-organ reflex sensitization between the bowels and the lower urinary tract. These findings further extend the role of activity-dependent AMPAR trafficking in viscero-visceral referred pain in the pelvis, making it a potential target for treatment of urogenital pain.

In *Aplysia*, Ht31-mediated disruption of PKA-AKAP binding blocks both short- and long-term synaptic plasticity.[36] Hippocampal memory has proven sensitive to pharmacologic blockage of PKA-AKAP interactions. Recent work has indicated that disruption of PKA anchorage in the mammalian amygdala by injection of Ht31 abolishes the naturally occurring plasticity required for auditory fear memory.[37] This evidence is in line with our results, which show that intrathecal application of Ht31 blocks capsaicin-dependent cross-organ reflex sensitization. Together, these data imply that altered PKA-AKAP assembly, which modulates intracellular PKA sites, may be a general mechanism that controls substrate phosphorylation during neural plasticity. Moreover, modulation of PKA anchorage may profoundly affect memory physiology in the brain and pain pathology in the spinal cord.

In the present study, we found that activation of PKA induces GluR1S845 phosphorylation accompanied by AMPAR membrane expression. In line with our findings, rapid phosphorylation of GluR1S845 has been shown to be required for NMDA receptor–induced AMPAR cell surface expression in human embryonic kidney cells.[6] Moreover, in cultured nucleus accumbens neurons, activation of dopaminergic D1 receptors promotes AMPAR insertion *via* a PKA-dependent pathway.[38] PKA activity plays important roles in both LTP and long-term depression, which are accompanied by changes in PKA-dependent phosphorylation of AMPAR subunits, indicating that PKA-dependent GluR1S845 phosphorylation may be critical to various forms of synaptic plasticity.[1,4] Regulation of the phosphorylation state of AMPAR is likely one of the earliest events associated with LTP and its expression.[39–41]

In recent years, a growing body of data has indicated that LTP and long-term depression are produced through the increase and removal of synaptic AMPARs, respectively.[1–5] GluR1S845 phosphorylation seems crucial in receptor trafficking–mediated synaptic plasticity in brain areas, as AMPAR trafficking and GluR1S845 phosphorylation have been correlated in brain neurons.[10] A recent *in vivo* study[42] demonstrated that mice with mutant GluR1 phosphorylation sites exhibit spatial memory deficits. Our present findings not only support the results of other researchers[42]—which provide a link between GluR1S845 phosphorylation and GluR1 trafficking–related neural plasticity in brain areas such as the hippocampus and cerebral cortex—but also extend the role of GluR1S845 phosphorylation in spinal neural plasticity, which is presumed to be the spinal mechanism that underlies viscero-visceral referred pain. However, we measured GluR1S845 expression in total lysate rather than membrane fraction. Thus, there is no direct evidence that AMPAR trafficking is dependent on GluR1S845 phosphorylation in the present study. The direct role of GluR1S845 phosphorylation in AMPA trafficking requires further investigation.

In summary, we propose that AKAP-dependent regulation of the AMPAR GluR1 subunit is initiated by the recruitment of GluR1 to AKAP, which results in the formation of the GluR1-AKAP complex, favoring basal phosphorylation of GluR1S845.[13] GluR1S845 phosphorylation caused by AKAP-anchored PKA might lead to GluR1 up-regulation in the cytoplasmic membrane, which enhances synaptic efficacy in the DH, resulting in reflex potentiation or sensitization.

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