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Second Messengers Mediating the Expression of Neuroplasticity in a Model of Chronic Pain in the Rat

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Abstract: Hyperalgesic priming is a model of the transition from acute to chronic pain, in which previous activation of cell surface receptors or direct activation of protein kinase C epsilon markedly prolongs mechanical hyperalgesia induced by pronociceptive cytokines. We recently demonstrated a role of peripheral protein translation, alpha-calmodulin-dependent protein kinase II (αCaMKII) activation, and the ryanodine receptor in the induction of hyperalgesic priming. In the present study, we tested if they also mediate the prolonged phase of prostaglandin E_2–induced hyperalgesia. We found that inhibition of αCaMKII and local protein translation eliminates the prolonged phase of prostaglandin E_2 hyperalgesia. Although priming induced by receptor agonists or direct activation of protein kinase C epsilon occurs in male but not female rats, activation of αCaMKII and the ryanodine receptor also produces priming in females. As in males, the prolonged phase of prostaglandin E_2–induced hyperalgesia in female rats is also protein kinase C epsilon–, αCaMKII-, and protein translation–dependent. In addition, in both male and female primed rats, the prolonged prostaglandin E_2–induced hyperalgesia was significantly attenuated by inhibition of MEK/ERK. On the basis of these data, we suggest that the mechanisms previously shown to be involved in the induction of the neuroplastic state of hyperalgesic priming also mediate the prolongation of hyperalgesia.

***Perspectives:** The data provided by this study suggest that direct intervention on specific targets may help to alleviate the expression of chronic hyperalgesic conditions.*

***Key words:** Second messengers, hyperalgesic priming, sensory neuron, mechanical hyperalgesia, rat.*

Hyperalgesic priming, a model of the transition from acute to chronic pain produced by a prior inflammatory insult, is expressed as a long-lasting neuroplastic state in which there is enhanced hyperalgesia induced by agents such as prostaglandin E_2 (PGE_2), adenosine, and serotonin.[3,38,41] The induction of hyperalgesic priming is triggered by a transient activation of protein kinase C epsilon (PKCε)[3,39] or molecules downstream of PKCε, including alpha-calmodulin-dependent protein kinase II (αCaMKII) and the ryanodine receptor,[20] inducing translation of mRNAs in the peripheral terminal of the nociceptor[19,20] that are responsible for the chronic maintenance of this state.[6,19]

In the naïve rat, PGE_2-induced hyperalgesia is of short duration (~1 hour) and dependent on activation of stimulatory G-protein (Gs) and protein kinase A.[1] However, in the primed state, the hyperalgesia induced by PGE_2 is markedly prolonged, lasting more than 24 hours,[3] due to a novel linkage of prostaglandin receptor activation to an additional signaling pathway involving an inhibitory G-protein (Gi), phospholipase C beta 3 (PLCβ3) and PKCε during the prolonged phase of the PGE_2-induced hyperalgesia.[3,15,22,28,32,37,41]

In this study, we show that the prolonged phase of PGE_2-induced hyperalgesia also involves αCaMKII, local protein translation, and the mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway, mechanisms not involved in signaling for PGE_2-induced hyperalgesia in the naïve state.[2] We also show that these mechanisms contribute to the prolonged phase of PGE_2-induced hyperalgesia in female rats in which hyperalgesic priming can be induced by αCaMKII or ryanodine receptor activation.

Methods

Animals

All experiments were performed on adult male and female Sprague Dawley rats (220–400 g; Charles River

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Laboratories, Wilmington, MA). Animals were housed, 3 per cage, under a 12-hour light/dark cycle in a temperature- and humidity-controlled room in the animal care facility of the University of California, San Francisco. Food and water were available ad libitum. All nociceptive testing was done between 10:00 AM and 5:00 PM. The experimental protocols were approved by the Institutional Animal Care and Use Committee at University of California, San Francisco, and adhered to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All effort was made to minimize the number of animals used and their suffering.

Mechanical Nociceptive Threshold Testing

Mechanical nociceptive threshold was quantified using an Ugo Basile Analgesymeter (Randall-Selitto paw-withdrawal test; Stoelting, Chicago, IL), which applies a linearly increasing mechanical force to the dorsum of the rat's hind paw, as previously described.[41,48,50] The nociceptive threshold was defined as the force in grams at which the rat withdrew its paw, and baseline paw-pressure threshold was defined as the mean of the 3 readings taken before the test agents were injected. Each paw was treated as an independent measure and each experiment was performed on a separate group of rats. Data are presented as mean change from baseline mechanical nociceptive threshold.

Drugs

The following drugs were used in this study: PGE_2, the ryanodine receptor activator ryanodine, and the specific inhibitor of MEK 1/2 U0126 (1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene),[13] all from Sigma-Aldrich (St. Louis, MO); alpha calcium/calmodulin-dependent protein kinase II recombinant (activated αCaMKII; New England Biolabs, Ipswich, MA), the CaMKII inhibitor peptide CaM2INtide (GenScript, Piscataway, NJ), $PKC\varepsilon V_{1\text{-}2}$, a PKCε-specific translocation inhibitor peptide (PKCε-I)[27,33] (Calbiochem, La Jolla, CA), the PKCε activator ψεRACK (Biomatik, Wilmington, DE), and the protein translation inhibitors cordycepin 5′-triphosphate sodium salt (Sigma-Aldrich) and rapamycin (EMD Chemicals, Gibbstown, NJ). The selection of the drug doses used in this study was based on our previous studies.[3,19,33,36,37,49]

Stock solutions of PGE_2 in absolute ethanol (1 μg/μL) were further diluted in .9% NaCl (1:50, C_{final} = .2 μg/μL) immediately before injection. The ethanol concentration of the final PGE_2 solution was ~2% and the injection volume 5 μL. Stock solutions of cordycepin (10 μg/μL, dissolved in a 1:1 mixture of .9% NaCl and absolute ethanol) or rapamycin (20 μg/μL, dissolved in absolute dimethyl sulfoxide) were further diluted in .9% NaCl or distilled water, respectively, immediately before injection. The ethanol or dimethyl sulfoxide concentration in the final solutions was ~2%.

Activation of αCaMKII was performed in vitro, and a dose of 25 ng, in a volume of 2.5 μL, of the activated αCaMKII was injected intradermally on the dorsum of the rat hind paw. αCaMKII was diluted in 1X NEBuffer for PK (50 mM Tris–HCl, 10 mM $MgCl_2$, .1 mM ethylenediaminetetraacetic acid, 2 mM dithiothreitol, .01% Brij 35, pH 7.5 at 25°C) supplemented with 200 μM adenosine triphosphate, 1.2 μM calmodulin, and 2 mM $CaCl_2$, and incubated for 10 minutes at 30°C before injection.

Drugs were administered intradermally on the dorsum of the hind paw via a beveled 30-gauge hypodermic needle attached to a Hamilton microsyringe (Hamilton Company, Reno, NV) by a short length of polyethylene (PE-10) tubing. The administration of all drugs, except PGE_2, was preceded by a hypotonic shock to facilitate cell permeability to these agents (2 μL of distilled water, separated by a bubble of air to avoid mixing in the same syringe), to get compounds into the nerve terminal.[7,9]

Oligodeoxynucleotide Antisense to αCaMKII

The oligodeoxynucleotide (ODN) antisense sequence for the α-subunit of CaMKII, 5′-GGT AGC CAT CCT GGC ACT-3′ (Invitrogen, Carlsbad, CA), was directed against a unique region of the rat messenger RNA (mRNA) sequence. The corresponding National Center for Biotechnology Information GenBank accession number and ODN position within the mRNA sequence are NM_012920 and 33 to 50, respectively. That this antisense can be used to downregulate the expression of αCaMKII has been shown previously.[11] The ODN mismatch[10] sequence 5′-GGT AGC CAT **AAG** GGC ACT-3′ corresponds to the antisense sequence with 3 bases mismatched.

Before use, the ODNs were lyophilized and reconstituted in .9% NaCl to a concentration of 2 μg/μL. During each injection, rats were briefly anesthetized with 2.5% isoflurane in 95% O_2. A 30-gauge hypodermic needle was inserted into the subarachnoid space on the midline, between the L4 and L5 vertebrae. A total of 40 μg ODN in a volume of 20 μL was slowly injected. Proper intrathecal injections were systematically confirmed by checking for a sudden flicking of the tail, a reflex that is evoked by subarachnoid space access and bolus injection.[35] The animals regained consciousness approximately 1 minute after the injection. The use of antisense to manipulate the expression of proteins in nociceptors, important for their role in nociceptor sensitization, is well supported by previous studies by others[40,45-47] as well as our group.[6,18,21,39]

Prolonged Phase of PGE_2-Induced Mechanical Hyperalgesia

To evaluate signaling mechanisms involved in the prolongation phase of the PGE_2-induced mechanical hyperalgesia observed in our model of chronic pain, we injected 1 of 3 agents that induce priming—ψεRACK, activated αCaMKII, or ryanodine—intradermally on the dorsum of the rat's hind paw.[3,20] These agents induce mechanical hyperalgesia that resolves in 3 to 5 days