eIF2α phosphorylation controls thermal nociception

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A response to environmental stress is critical to alleviate cellular injury and maintain cellular homeostasis. Eukaryotic initiation factor 2 (eIF2) is a key integrator of cellular stress responses and an important regulator of mRNA translation. Diverse stress signals lead to the phosphorylation of the α subunit of eIF2 (Ser51), resulting in inhibition of global protein synthesis while promoting expression of proteins that mediate cell adaptation to stress. Here we report that eIF2α is instrumental in the control of noxious heat sensation. Mice with decreased eIF2α phosphorylation (*eIF2α*$^{+/S51A}$) exhibit reduced responses to noxious heat. Pharmacological attenuation of eIF2α phosphorylation decreases thermal, but not mechanical, pain sensitivity, whereas increasing eIF2α phosphorylation has the opposite effect on thermal nociception. The impact of eIF2α phosphorylation (p-eIF2α) on thermal thresholds is dependent on the transient receptor potential vanilloid 1. Moreover, we show that induction of eIF2α phosphorylation in primary sensory neurons in a chronic inflammation pain model contributes to thermal hypersensitivity. Our results demonstrate that the cellular stress response pathway, mediated via p-eIF2α, represents a mechanism that could be used to alleviate pathological heat sensation.

pain | eIF2α | cellular stress response pathway | TRPV1

Response to stress is a major cellular function involved in many physiological and pathological conditions. Cells respond to various forms of stress by activating specific molecular cascades that orchestrate antistress responses or induce apoptosis (1). A key effector of cellular stress responses is the eukaryotic initiation factor 2 (eIF2) (2). Phosphorylation of eIF2 causes a reduction in global translation, allowing cells to conserve energy and modify gene expression to effectively manage stress conditions. Diverse stress signals converge onto eIF2 to integrate stress responses through phosphorylation of the α subunit of eIF2.

eIF2 binds GTP and the initiator methionyl-tRNA (Met-tRNA$_i$) to form the ternary complex (eIF2-GTP-Met-tRNA$_i$). The ternary complex binds the small ribosomal subunit to form the ribosomal preinitiation complex, which scans the 5′UTR of the mRNA for the start codon to initiate mRNA translation (3). On engagement of the initiation codon, GTP is hydrolyzed to GDP (4). The recycling of inactive GDP-bound eIF2 to the active GTP-bound form is catalyzed by the guanine nucleotide exchange factor, eIF2B. Phosphorylation of the α subunit of eIF2 at serine 51 converts eIF2 from a substrate to a competitive inhibitor of eIF2B (4). Because the amount of eIF2B is lower than eIF2, phosphorylation of a small fraction of the eIF2 in the cell is sufficient to strongly inhibit eIF2B activity and translation initiation.

eIF2α is phosphorylated by four eIF2α kinases, each activated in a different stress condition (5–7). PKR (double-stranded RNA-dependent protein kinase) is activated by double-stranded RNA during viral infection; PERK (PKR-like ER kinase) by endoplasmic reticulum stress; GCN2 (general control non–derepressible-2) by nutrient deprivation and UV light; and HRI (heme-regulated inhibitor) by heme deficiency. The eIF2α kinases, except for HRI, are prominently expressed in the mammalian nervous system (8).

Phosphorylation of eIF2α blocks general translation but paradoxically stimulates translation of mRNAs that contain upstream ORFs (uORFs) in their 5′ UTR, such as ATF4 [a cAMP-response element binding protein 2 (CREB-2)] and CHOP (a proapoptotic transcription factor) (9). ATF4 enhances the expression of a related transcription factor, ATF3, which together with ATF4 contribute to stress adaptation by regulating genes involved in metabolism, the cellular redox status, and apoptosis (10, 11). In neurons, an activity-dependent decrease in eIF2α phosphorylation augments long-term potentiation (LTP) and memory via suppression of ATF4 expression (12). Conversely, up-regulation of p-eIF2α is associated with long-term depression (LTD) (13, 14) and several pathophysiological conditions including viral infection, inflammation, and neurodegeneration (15–18). Elevated phosphorylation of eIF2α has been documented in the brain of aged animals (19) and Alzheimer's disease patients and model mice (20, 21). Normalization of p-eIF2α in Alzheimer's disease model mice rescued deficits in protein synthesis, synaptic plasticity, and spatial memory (22). Additionally,

Significance

Distinct cellular stresses converge on the translation initiation factor, eukaryotic initiation factor 2α (eIF2α) to modulate the rate of protein synthesis. Increased phosphorylation of eIF2α has been described in peripheral neurons from neuropathic and diabetic rats. However, the role of eIF2α phosphorylation in pain has not been reported. Here we show that phosphorylation of eIF2α controls thermal, but not mechanical, sensation via modulation of the activity of a major heat transducer, transient receptor potential vanilloid 1. We also find that chronic inflammation-induced eIF2α phopshorylation contributes to inflammation-induced thermal hypersensitivity. These results demonstrate that eIF2α phosphorylation plays a major role in controlling noxious heat sensitivity.

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phosphorylation of eIF2α is associated with synaptic deficits and neuronal loss in prion-disease model mice (23).

The role of eIF2 in the pain pathway is unknown. An endoplasmic reticulum (ER) stress response is induced in the peripheral nervous system of type I diabetic rats, as phosphorylation of PERK and eIF2α, along with other ER stress markers, is up-regulated (24). Induction of ER stress is accompanied by hypersensitivity, and attenuation of ER stress by an inhibitor of soluble epoxide hydrolase (sEH) down-regulated ER stress markers, p-PERK, p-eIF2α, and reversed mechanical hypersensitivity (24). Despite these intriguing observations, a direct link between eIF2α phosphorylation and nociception has not been established. Using a transgenic mouse model with reduced phosphorylation (by ∼50%) of eIF2α ($eIF2\alpha^{+/S51A}$), we show that p-eIF2α controls thermal, but not mechanical, sensitivity via the regulation of transient receptor potential vanilloid receptor 1 (TRPV1) activity. Moreover, we find that eIF2α phosphorylation is induced in primary nociceptors in a chronic inflammation model and that it contributes to inflammatory pain hypersensitivity.

Results

p-eIF2α Is Increased in Dorsal Root Ganglia After Chronic Inflammation. First, we examined the distribution of eIF2α and its phosphorylated form, p-eIF2α, in dorsal root ganglia (DRGs) and spinal cord. Immunostaining revealed neuronal expression of eIF2α and p-eIF2α in peptidergic (CGRP-positive), nonpeptidergic (IB4-positive), TRPV1-positive small diameter, and NF200-positive large diameter neuronal cell bodies in DRGs (Fig. 1 *A* and *B*). Colocalization analysis showed that 19.7% of p-eIF2α–positive cells express CGRP, 36.6% express IB4, 20.8% express TRPV1, and 47% express NF200 (Fig. 1*B*, *Bottom*). In the dorsal horn of the spinal cord, eIF2α and p-eIF2α were found in neurons, as they colocalized with the neuronal marker NeuN, but not with the astrocyte marker, glial fibrillary acidic protein (GFAP) (Fig. S1). However, in the spinal cord p-eIF2α signal was rather weak and detected only in a small fraction of NeuN-positive neurons (Fig. S1).

To determine whether phosphorylation of eIF2α is affected by chronic inflammation, we injected complete Freund's adjuvant (CFA) s.c. into the mouse hind paw (intraplantar injection) and measured the levels of p-eIF2α in lumbar DRGs and dorsal horn of the spinal cord. Levels of p-eIF2α were increased in DRGs, but not in the spinal cord, 1 d after the onset of inflammation, decreased subsequently, and returned to normal after 10 d (Fig. 1*C*). The alterations in p-eIF2α concurred with the inflammation-induced changes in thermal and mechanical thresholds (Fig. 1*D*), raising the possibility that the increase in eIF2α phosphorylation mediates the inflammatory hypersensitivity.

P-eIF2α Controls Thermal Sensitivity. Having established that p-eIF2α is increased in DRGs in response to chronic inflammation, we investigated its role in nociception. To this end, we used a transgenic knock-in (KI) mouse model (25), in which serine-51 is mutated to a nonphosphorylatable alanine residue in one allele ($eIF2\alpha^{+/S51A}$, homozygous KI mice are not viable), leading to a ∼50% reduction in basal eIF2α phosphorylation (Fig. 2*A*). Mechanical sensitivity in the von Frey and tail clip tests did not differ between $eIF2\alpha^{+/S51A}$ mice and their WT littermates (Fig. 2*B*). However, thermal withdrawal and nocifensive behavior latencies were significantly prolonged in $eIF2\alpha^{+/S51A}$ mice compared with WT mice in the radiant heat paw withdrawal, hot water tail withdrawal, and hot-plate tests (40.2 ± 9.7%, 44.0 ± 13.1%, and 21.6 ± 6.6% increase, respectively; Fig. 2*C*), indicating reduced sensitivity to noxious heat in $eIF2\alpha^{+/S51A}$ mice. No difference in sensitivity to noxious cold was observed between WT and $eIF2\alpha^{+/S51A}$ mice (Fig. 2*D*). $eIF2\alpha^{+/S51A}$ mice also exhibited reduced inflammatory pain in the formalin test. Nocifensive (licking/shaking) behavior was significantly reduced (by 31.9 ± 6.0%) in $eIF2\alpha^{+/S51A}$ mice during the late/tonic phase (10–60 min after formalin injection), compared

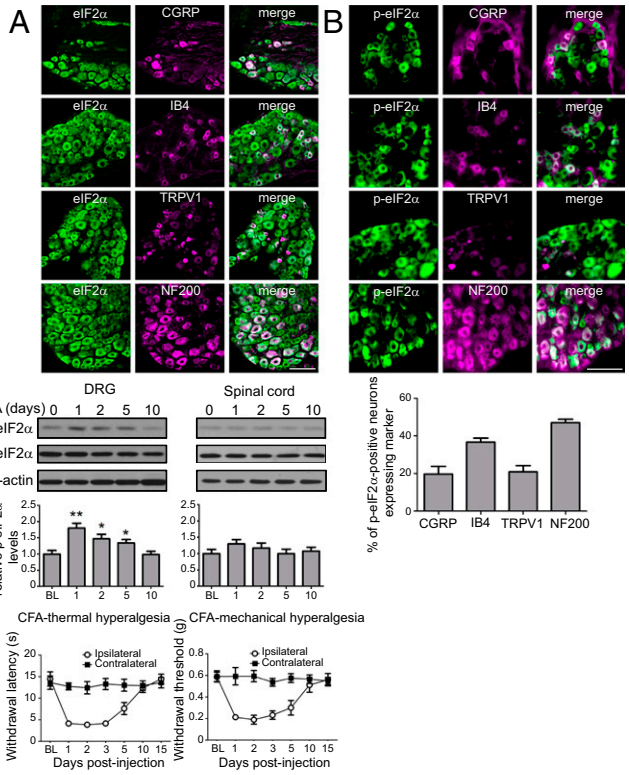

Fig. 1. eIF2α is expressed in DRG neurons and its phosphorylation is increased in an inflammatory pain model. The distribution of total and p-eIF2α in mouse lumbar DRG was examined using immunostaining. Total (*A*) and p-eIF2α (B) were costained with CGRP, IB4, TRPV1, and NF200. Percent of p-eIF2α–positive neurons expressing the markers is shown in *B* (*Bottom*). (*C*) Mice were injected (intraplantar) with CFA, and levels of eIF2α phosphorylation were measured in DRGs at different time points after injection using Western blot analysis (n = 4 mice per condition). (*D*) CFA induces thermal (*Left*) and mechanical (*Right*) hypersensitivity as assessed in radiant heat paw withdrawal and von Frey assays, respectively (n = 5 males and 4 females per assay). Data are presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ by Bonferroni post hoc test following one-way ANOVA. (Scale bar, 100 μm.) For distribution of eIF2α and p-eIF2α in the spinal cord, see Fig. S1.

with WT mice, whereas no differences were found in the early/acute phase (0–10 min) between these groups (Fig. 2*E*). The behavioral differences occurred despite equal degrees of paw edema in the two genotypes (Fig. 2*E*). Taken together, these results demonstrate that p-eIF2α is up-regulated in DRGs in response to chronic inflammation, and mice with reduced eIF2α phosphorylation exhibit decreased heat sensitivity but not mechanical sensitivity.

As eIF2α is phosphorylated by four different kinases (Fig. 3*A*), it was pertinent to study the effect of each kinase on p-eIF2α and thermal threshold. Because HRI expression is very low in the nervous system (12), we examined sensitivity to noxious heat in $Perk^{+/-}$ ($Perk^{-/-}$ exhibits severe postnatal growth retardation) (26), $Pkr^{-/-}$, and $Gcn2^{-/-}$ mice. *Perk* heterozygous had reduced p-eIF2α in DRGs and decreased noxious heat sensation (43.0 ±7.7% increase in latency to withdrawal in the radiant heat paw withdrawal test; Fig. 3*B*). Mechanical thresholds in the von Frey test were not altered in $Perk^{+/-}$ mice, similar to $eIF2\alpha^{+/S51A}$ mice. $Gcn2^{-/-}$ and $Pkr^{-/-}$ mice did not display a significant reduction in p-eIF2α level and sensitivity to noxious heat (Fig. 3 *C* and *D*); however, double KO mice for *Gcn2* and *Pkr* (*Gcn2*/*Pkr* DKO) exhibited reduced p-eIF2α levels (Fig. 3*C*) and elevated thermal thresholds (Fig. 3*E*). This finding suggests a redundant role for these two kinases.

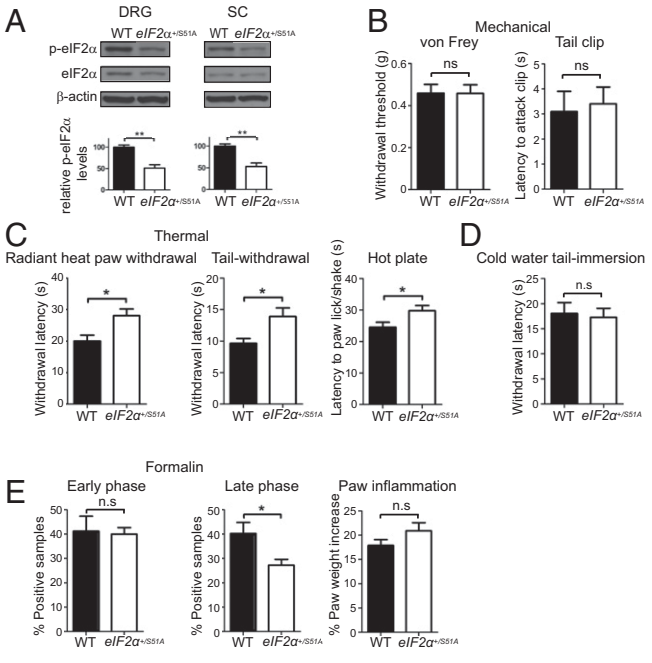

Fig. 2. Noxious heat sensation is reduced in *eIF2α*$^{+/S51A}$ mice. (*A*) eIF2α phosphorylation is reduced in DRGs and spinal cord of *eIF2α*$^{+/S51A}$ mice. *eIF2α*$^{+/S51A}$ mice demonstrate no alterations in mechanical sensitivity (*B*; n = 4 males and 4 females per genotype, $P > 0.05$), whereas noxious heat sensation is significantly attenuated (*C*; $n = 4$ males and 4 females per genotype per assay, $P < 0.05$). (*D*) Sensitivity to cold is not changed in *eIF2α*$^{+/S51A}$ mice ($n = 4$ males and 6 females per genotype). (*E*) Nocifensive (licking/shaking) behavior is significantly reduced in formalin test during the late/tonic phase (10–60 min after formalin injection, $P < 0.05$), whereas no differences are found in the early/acute phase ($n = 4$ males and 4 females per genotype). Changes in paw weight, indicative of formalin-induced inflammation, are not different in *eIF2α*$^{+/S51A}$ mice (*E*, *Right*; $P > 0.05$). Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$; ns, not significant by Student *t* test.

Next, we examined whether modulation of eIF2α phosphorylation by drugs alters the thermal threshold. eIF2α phosphorylation was decreased by an inhibitor of eIF2α kinase, PKR (PKRi) (27). i.p. administration of PKRi over 3 d reduced noxious heat sensation in a dose-dependent manner, as indicated by the increased withdrawal latency in the radiant heat paw withdrawal test (Fig. 3*F*), with no effect on mechanical threshold. Conversely, when eIF2α phosphorylation was increased by i.p. administration of Sal003, an inhibitor of the eIF2α phosphatase complex, GADD34/PP1 (growth arrest and DNA-damage-inducible 34/protein phosphatase1) (28) (Fig. 3*A*), thermal thresholds were decreased (Fig. 3 *G* and *H*), whereas mechanical thresholds were not affected. In summary, using genetic and pharmacological approaches, we show that decreasing eIF2α phosphorylation reduces noxious heat sensation, whereas increasing p-eIF2α levels engenders the opposite effect.

TRPV1 Activity Mediates the Effect of Reduced p-eIF2α on Thermal Thresholds. The strikingly specific impact of eIF2α phosphorylation on noxious heat sensation suggests that mechanisms controlling heat transduction might be selectively controlled. TRPV1 channels transduce noxious heat and are also implicated in inflammation-induced thermal hypersensitivity (29). TRPV1 activity is tightly regulated via gene expression and posttranslational mechanisms (30). We examined the possibility that TRPV1 mediates the effect of eIF2α phosphorylation on heat sensation by studying the impact of PKRi and Sal003 on thermal thresholds in *Trpv1*$^{-/-}$ mice (29). PKRi increased thermal threshold in WT mice, but not in *Trpv1*$^{-/-}$ mice (Fig. 4*A*). Conversely, Sal003 decreased thermal threshold in WT mice, but not in *Trpv1*$^{-/-}$ mice (Fig. 4*B*). These data demonstrate that eIF2α phosphorylation controls thermal threshold in a TRPV1-dependent manner. To assess TRPV1 activity, we recorded TRPV1-dependent currents in sensory neurons from *eIF2α*$^{+/S51A}$ and WT mice. Capsaicin, a specific TRPV1 agonist, elicited significantly smaller currents in dissociated DRG neurons prepared from *eIF2α*$^{+/S51A}$ compared with WT mice (92% decrease in *eIF2α*$^{+/S51A}$ neurons; Fig. 4*C*). For whole-cell recordings, small-diameter (<30 μm) neurons were selected, and only capsaicin-sensitive neurons (~30% of all tested neurons in WT and *eIF2α*$^{+/S51A}$ groups) were included in the analysis. Resting membrane potential (V_{rest}), input resistance (R_{in}), and membrane capacitance (C_m) were not different between WT and *eIF2α*$^{+/S51A}$ neurons (WT V_{rest} −49.74 ± 3.41 mV, *eIF2α*$^{+/S51A}$ V_{rest} −46.79 ± 3.99 mV, $P = 0.584$; WT R_{in} 686.21 ± 117.41 MΩ, *eIF2α*$^{+/S51A}$ R_{in} 517.13 ± 38.41 MΩ, $P = 0.193$; WT

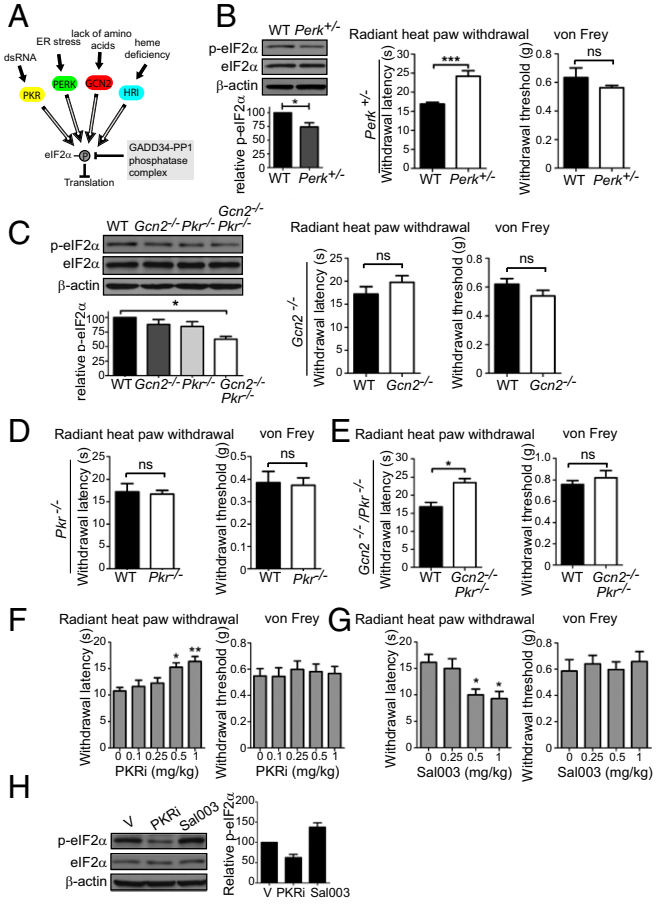

Fig. 3. eIF2α kinases control thermal threshold. (*A*) eIF2α kinases and phosphatase GADD34/PP1 (growth arrest and DNA-damage-inducible 34/protein phosphatase1). (B) *Perk*$^{+/-}$ mice show a decrease in p-eIF2α ($n = 4$ mice per genotype, $P < 0.05$) and in noxious heat sensitivity ($n = 4$ males and 4 females per genotype, $P < 0.001$). *Gcn2*$^{-/-}$ and *Pkr*$^{-/-}$ mice show no alteration in thermal latencies (*C* and *D*, respectively; $n = 4$ males and 4 females per genotype, $P > 0.05$). (*E*) *Gcn2*$^{-/-}$ *Pkr*$^{-/-}$ double KO mice show reduced noxious heat sensitivity ($n = 3$ males and 4 females per genotype, $P < 0.05$). eIF2α phosphorylation in DRGs of *Gcn2*$^{-/-}$, *Pkr*$^{-/-}$, *and Gcn2*$^{-/-}$ *Pkr*$^{-/-}$ double KO mice is shown in *C* ($n = 3$ mice per genotype). PKR inhibitor (PKRi) (*F*) and Sal003 (*G*) were injected i.p. for 3 d daily, and thermal and mechanical thresholds were measured 30 min after the last injection ($n = 4$ males and 4 females per condition). (*H*) eIF2α phosphorylation in DRGs of mice injected with PKRi (1 mg/kg) and Sal003 (1 mg/kg) is shown ($n = 5$ mice per drug). Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant by Student *t* test and Bonferroni post hoc test following one-way ANOVA.

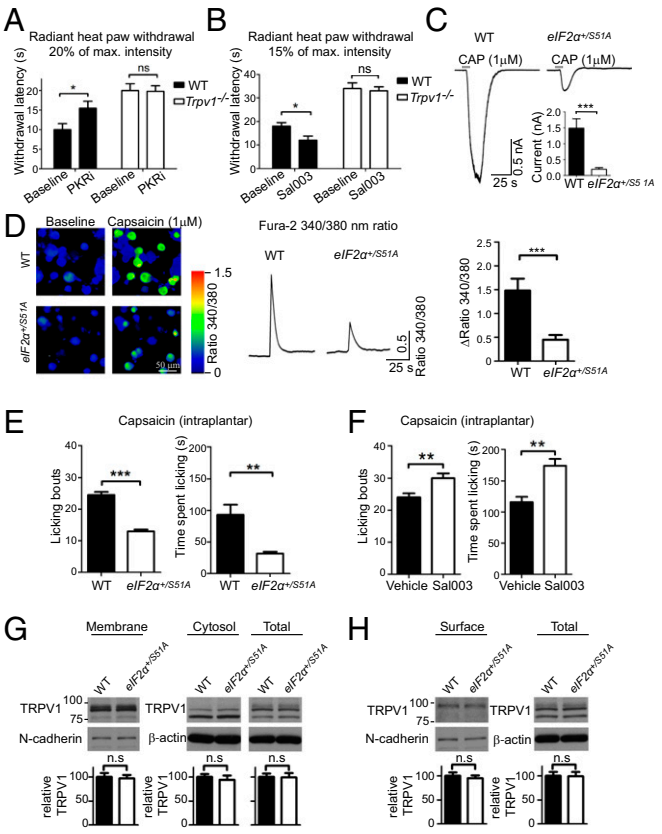

Fig. 4. eIF2α phosphorylation regulates thermal threshold via TRPV1. Modulation of eIF2α phosphorylation in *Trpv1*$^{-/-}$ mice does not alter heat sensation. PKRi (1 mg/kg for 3 d daily, i.p.) elevates thermal threshold in WT but not in *Trpv1*$^{-/-}$ mice (*A*; $n = 4$ males and 4 females per genotype-drug condition). Sal003 (1 mg/kg for 3 d daily, i.p.) decreases thermal threshold in WT but not in *Trpv1*$^{-/-}$ mice (*B*, $n = 4$ males and 4 females per genotype-drug condition). For the radiant heat paw withdrawal test, the light beam was set to 20% of the maximal intensity in *A* and to 15% in *B*. Capsaicin (1 μM) evokes smaller currents (*C*, $n = 12$ cells for *eIF2α*$^{+/S51A}$ and 10 for WT, from three different neuronal cultures per genotype) and smaller calcium transients (*D*, $n = 72$ cells for *eIF2α* $^{+/S51A}$ and $n = 46$ cells for WT, from four different neuronal cultures per genotype, using Fura-2 340/380-nm ratio) in cultured DRG neurons prepared from *eIF2α*$^{+/S51A}$ compared with WT mice. Capsaicin (2.5 μg), injected s.c. into the plantar surface of the hindpaw, elicited less nocifensive behaviors in *eIF2α*$^{+/S51A}$ compared with WT mice (*E*; $n =$ 4 males and 4 females per genotype or drug), whereas in mice injected with Sal003 (1 mg/kg for 3 d daily, i.p.) capsaicin-induced pain behavior is increased (*F*). Western blot analysis shows that the TRPV1 protein levels are not altered in membrane and cytosolic fractions, as well as in total lysates of DRGs of *eIF2α*$^{+/S51A}$ mice (*G*; $n = 4$ mice and genotype). TRPV1 surface levels were measured in DRG cultured neurons prepared from *eIF2α*$^{+/S51A}$ and WT mice using surface biotinylation assay (*H*; $n = 3$/group). Data are presented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ by Student *t* test and Student *t* test following two-way (genotype × drug) ANOVA.

C_m 13.67 ± 2.18 pF, *eIF2α*$^{+/S51A}$ C_m 14.68 ± 1.95 pF, $P = 0.735$, $n = 8$/genotype). Moreover, using calcium imaging, we observed smaller capsaicin-induced calcium transients in cultured *eIF2α*$^{+/S51A}$ DRG neurons compared with WT neurons (Fig. 4*D*). The cell body diameter of the responding neurons was not different between the two genotypes (WT 19.18 ± 0.45 μm, $n =$ 53, *eIF2α*$^{+/S51A}$ 19.32 ± 0.39 μm, $n = 88$, $P = 0.81$). Consistent with these results, intraplantar s.c. administration of capsaicin induced significantly less nocifensive behavior in *eIF2α*$^{+/S51A}$ compared with WT mice (Fig. 4*E*). Conversely, mice with high p-eIF2α levels, following Sal003 injections, exhibited increased nociceptive responses to capsaicin (Fig. 4*F*). Despite the reduction in the amplitude of TRPV1-mediated currents in *eIF2α*$^{+/S51A}$ neurons, Western blot analysis showed that protein levels of TRPV1 in cytosolic and membrane fractions of DRG lysates from *eIF2α*$^{+/S51A}$ mice were not changed compared with WT mice (Fig. 4*G*). To examine whether trafficking of TRPV1 to the cell surface is affected by *eIF2α* phosphorylation, we used a surface biotinylation assay followed by Western blot analysis of TRPV1. We found no differences in TRPV1 amounts on the cell surface (Fig. 4*H*), indicating that TRPV1 activity, but not protein levels or trafficking to the plasma membrane, is reduced in *eIF2α*$^{+/S51A}$ neurons. Taken together, these data indicate that TRPV1 is an important mediator of the effect of p-eIF2α on the thermal threshold and suggest that TRPV1 activity is modulated by eIF2α phosphorylation.

PKR-Mediated eIF2α Phosphorylation Contributes to Thermal Hypersensitivity. After establishing that eIF2α phosphorylation regulates thermal sensation, we studied whether CFA-induced increase in p-eIF2α contributes to thermal hypersensitivity. eIF2α kinase, PKR, has been implicated in inflammatory responses (31). Thus, we assessed PKR activation in DRGs following CFA injection and found that levels of p-PKR were significantly increased (Fig. 5*A*), raising the possibility that the increase in p-eIF2α is mediated via PKR activation. Consequently, we assessed thermal and mechanical hypersensitivity of *Pkr*$^{-/-}$ mice after CFA-induced inflammation. *Pkr*$^{-/-}$ mice exhibited reduced thermal,

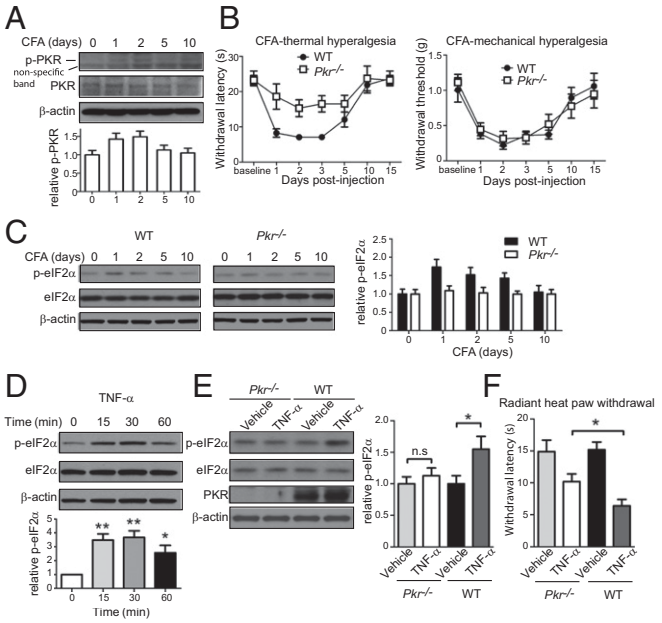

Fig. 5. TNF-α–mediated PKR activation elevates p-eIF2α. (*A*) Phosphorylation of PKR (Thr451) was measured at different time points after CFA injection ($n = 4$ mice). (*B*) CFA-induced thermal hyperalgesia is attenuated in *Pkr*$^{-/-}$ mice compared with WT mice, whereas mechanical hyperalgesia is not changed ($n = 4$ males and 4 females per genotype). (*C*) P-eIF2α in DRGs was measured at different time points after CFA injection in WT and *Pkr*$^{-/-}$ mice. (*D*) HEK293 cells were treated with TNF-α (100 ng/mL) for 15, 30, and 60 min, and p-eIF2α was measured ($n = 3$, $P < 0.05$). (*E*) TNF-α (20 ng in 5 μL PBS + 0.5% BSA) was injected i.t. three times every 3 h in WT and *Pkr*$^{-/-}$ mice, and the p-eIF2α was measured in lumbar DRGs 30 min after the last injection. TNF-α induced an increase in p-eIF2α in WT but not in *Pkr*$^{-/-}$ mice ($n = 5$ females per genotype per drug). (*F*) TNF-α (20 ng, i.t., three injections at 3-h intervals) elicited bigger thermal hyperalgesia in WT mice compared with *Pkr*$^{-/-}$ mice ($n = 4$ males and 4 females per genotype per drug, $P < 0.05$). Data are presented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ by Bonferroni post hoc test following one-way ANOVA and Student *t* test following two-way (genotype × drug) ANOVA.

but not mechanical, pain hypersensitivity after CFA injection (Fig. 5*B*). Moreover, p-eIF2α increased after CFA injection in DRGs of WT mice, but not in $Pkr^{-/-}$ mice (Fig. 5*C*). These data demonstrate that PKR is required for the up-regulation of p-eIF2α following CFA and contributes to thermal hyperalgesia.

TNF-α induces a robust up-regulation of eIF2α phosphorylation in cultured cells and in the nervous system via activation of PKR (32–36). Because TNF-α is a major proinflammatory cytokine (37–40), which plays a critical role in the pathogenesis of inflammatory pain (41, 42), we examined whether inflammation-induced TNF-α promotes eIF2α phosphorylation. First, we showed that TNF-α elevates p-eIF2α in HEK293 cells, replicating previous studies (Fig. 5*D*). Importantly, intrathecally delivered TNF-α increased p-eIF2α in DRGs of WT mice, but not $Pkr^{-/-}$ mice (Fig. 5*E*), supporting the idea that TNF-α stimulates p-eIF2α via PKR. In accordance with previous reports, TNF-α (i.t.) induced heat hyperalgesia in WT mice (37), whereas in $Pkr^{-/-}$ mice this hyperalgesia was significantly attenuated (Fig. 5*F*). Taken together, these results demonstrate that TNF-α- and PKR-dependent eIF2α phosphorylation contributes to chronic inflammation-induced thermal hypersensitivity.

Discussion

Here we describe a previously unrecognized role for the cellular stress response pathway in nociception. Transgenic mice with decreased eIF2α phosphorylation ($eIF2\alpha^{+/S51A}$) exhibited reduced responses to noxious heat and attenuated nocifensive behavior in the late phase of formalin test, whereas cold sensitivity and mechanical thresholds were not altered. The noxious heat-specific phenotype was recapitulated in transgenic mice in which the eIF2α kinases PERK and PKR/GCN2 were reduced or knocked out, as well as in response to pharmacological manipulation of eIF2α phosphorylation. Reducing eIF2α phosphorylation with a PKR inhibitor attenuated noxious heat sensitivity, whereas increasing eIF2α phosphorylation with Sal003 had the opposite effect. Our findings indicate that the effect of p-eIF2α on thermal nociception is mediated via modulation of TRPV1 activity. First, we show that pharmacological modulation of eIF2α phosphorylation altered noxious heat sensation in WT mice, but had no effect in mice lacking TRPV1. Second, capsaicin-induced TRPV1-mediated currents and pain behavior were greatly reduced in $eIF2\alpha^{+/S51A}$ mice. Taken together, these data demonstrate that the eIF2α pathway controls noxious heat sensation via TRPV1. Because we found no evidence of alterations in TRPV1 protein levels or trafficking to the membrane, we postulate that the mechanism by which eIF2a phosphorylation affects thermal sensation involves modulation of TRPV1 activity. Under inflammatory conditions, TRPV1 can be sensitized by numerous inflammatory mediators (e.g., bradykinin, nerve growth factor, prostaglandins, serotonin, and histamine), via multiple agonists and modulators [protein kinase A (PKA), protein kinase C (PKC), metabolites of the cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome-P450 (CYP)-pathways, phospholipids, protons, and heat, among others], leading to the reduction in the activation threshold and eventually to pain hypersensitivity (43, 44). The modulators, which mediate the sensitization of TRPV1 activity by p-eIF2α, remain to be determined. eIF2α phosphorylation promotes translation of ATF4 mRNA (9, 11). Interestingly, ATF4 is increased in DRGs following facet joint distraction (45). Moreover, ATF4 transcriptionally activates ATF3 expression, which is a well-known cellular marker of nerve injury (46). This evidence suggests that some of the effects of p-eIF2α on thermal thresholds could be mediated by ATF4/ATF3 axis.

We show that TNF-α induces p-eIF2α in WT but not in $Pkr^{-/-}$ mice, indicating that TNF-α up-regulates p-eIF2α via PKR. PKR is activated after CFA injection, suggesting that TNF-α–mediated activation of PKR contributes to p-eIF2α up-regulation and thermal hypersensitivity. Our results do not exclude the involvement of other eIF2α kinases. For example, ER stress induces a robust PERK activation (19), which has a strong effect on eIF2α phosphorylation and thermal sensitivity (Fig. 3*B*).

A recent study found that hyperglycemia, activation of unfolded protein response (UPR), or dysregulation of calcium homeostasis induce ER stress in primary sensory neurons, as evident by the activation of PERK (and eIF2α), inositol-requiring enzyme 1a (IRE1a), ATF6, MAPK (p38 and JNK), and autophagy (LC3) (24). Whether the ER stress-induced mechanical pain is caused by elevated p-eIF2α or through other mechanisms was not documented. Because elevated p-eIF2α affects thermal, but not mechanical thresholds, it seems unlikely that the effects of ER stress on nociception are mediated via p-eIF2α, but could be attributed to the activation of p38 and JNK or to other ER stress-dependent mechanisms. Increased p-eIF2α was also documented in the sciatic nerve of rats with presumed neuropathic pain (47); however, the impact of this phosphorylation event on nociception has not been investigated.

Recent preclinical studies concluded that modulators of eIF2α phosphorylation might have therapeutic potential in treatment of several cellular stress-related pathologies such as Alzheimer's disease (22, 48), prion diseases (23), diabetes (49), Huntington's disease (50), and amyotrophic lateral sclerosis (51). It will be important to consider the effect of eIF2α phosphorylation on thermal nociception while developing clinically applicable compounds to the latter maladies.

In summary, we uncovered a previously unknown role for the cellular stress response pathway in nociception. This knowledge can be used to develop strategies to treat conditions associated with altered heat sensation, most notably burn pain, and should be considered while introducing eIF2α modulators to clinical practice.

Experimental Procedures

Behavioral Experiments, Electrophysiological Recordings, and Calcium Imaging. See *SI Experimental Procedures* for details of the experimental procedures. All procedures complied with Canadian Council on Animal Care guidelines and were approved by McGill University's Downtown Animal Care Committee.

Drugs. PKR inhibitor (PKRi) and Sal003 were purchased from Calbiochem and dissolved in 30% polyethylene glycol in saline. Capsaicin was purchased from Sigma and dissolved in ethanol. TNF-α was purchased from Kamiya Biomedical.

Western Blotting and Immunohistochemistry. Proteins were resolved on SDS-polyacrylamide gels using standard techniques. See *SI Experimental Procedures* for details of the experimental procedures and antibodies used.

Statistical Analyses. All results are expressed as mean ± SEM. All statistical comparisons were made with either the Student *t* test or a one-way ANOVA followed by between-group comparisons using the Bonferroni post hoc test, unless otherwise indicated, with $P < 0.05$ as the significance criterion. Power analyses were not possible because we had no a priori expectation of effect size but rather were informed by normative practices in the pain field (52).

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