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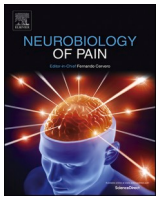

Original Research

ATP-gated potassium channels contribute to ketogenic diet-mediated analgesia in mice

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ABSTRACT

Chronic pain is a substantial health burden and options for treating chronic pain remain minimally effective. Ketogenic diets are emerging as well-tolerated, effective therapeutic strategies in preclinical models of chronic pain, especially diabetic neuropathy. We tested whether a ketogenic diet is antinociceptive through ketone oxidation and related activation of ATP-gated potassium (K_{ATP}) channels in mice. We demonstrate that consumption of a ketogenic diet for one week reduced evoked nocifensive behaviors (licking, biting, lifting) following intraplantar injection of different noxious stimuli (methylglyoxal, cinnamaldehyde, capsaicin, or Yoda1) in mice. A ketogenic diet also decreased the expression of p-ERK, an indicator of neuronal activation in the spinal cord, following peripheral administration of these stimuli. Using a genetic mouse model with deficient ketone oxidation in peripheral sensory neurons, we demonstrate that protection against methylglyoxal-induced nociception by a ketogenic diet partially depends on ketone oxidation by peripheral neurons. Injection of tolbutamide, a K_{ATP} channel antagonist, prevented ketogenic diet-mediated antinociception following intraplantar capsaicin injection. Tolbutamide also restored the expression of spinal activation markers in ketogenic diet-fed, capsaicin-injected mice. Moreover, activation of K_{ATP} channels with the K_{ATP} channel agonist diazoxide reduced pain-like behaviors in capsaicin-injected, chow-fed mice, similar to the effects observed with a ketogenic diet. Diazoxide also reduced the number of p-ERK^{+} cells in capsaicin-injected mice. These data support a mechanism that includes neuronal ketone oxidation and activation of K_{ATP} channels to provide ketogenic diet-related analgesia. This study also identifies K_{ATP} channels as a new target to mimic the antinociceptive effects of a ketogenic diet.

Introduction

Chronic pain negatively impacts the quality of life in 18–20% of American adults (Pitcher et al., 2019, Yong et al., 2022), and emerges from various etiologies, including diabetic peripheral neuropathy (DPN). Therapeutic options for patients suffering from chronic pain are limited to medications with limited efficacy and serious complications. Many molecular mechanisms are proposed to contribute to pain in DPN, including inflammation (Bekircan-Kurt et al., 2014), sensitization of transient receptor potential cation (TRP) channels TRPA1 and TRPV1 (Eberhardt et al., 2012, Bestall et al., 2018, Hiyama et al., 2018, Lam et al., 2018, Griggs et al., 2019), and accumulation of reactive metabolites such as methylglyoxal (Bierhaus et al., 2012, Eberhardt et al., 2012, Huang et al., 2016, Düll et al., 2019, Griggs et al., 2019). Recent work from our group and others has identified very low-carbohydrate, ketogenic diets as a promising therapeutic strategy in preclinical models of diabetic peripheral neuropathy and chronic pain (Ruskin et al., 2009, Cooper et al., 2018b, Di Lorenzo et al., 2019, Bongiovanni et al., 2021, Ruskin et al., 2021, Zhong et al., 2021, Enders et al., 2022a, Enders et al., 2022b). One mechanism by which a ketogenic diet reduces

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nociception through detoxification of methylglyoxal (Enders et al., 2022b). Methylglyoxal causes pain and nociception in humans and rodents by directly activating TRPA1 (Eberhardt et al., 2012, Andersson et al., 2013, Griggs, Laird et al. 2017, Düll et al., 2019, Griggs et al., 2019). Methylglyoxal detoxification does not, however, completely account for the analgesic action of a ketogenic diet, as ketogenic diets improve nociception in pain models not associated with elevated methylglyoxal (Ruskin et al., 2009, Di Lorenzo et al., 2019, Bongiovanni et al., 2021, Ruskin et al., 2021, Zhong et al., 2021). Here, we investigated alternative mechanisms that could contribute to the analgesic effects of a ketogenic diet.

ATP-gated potassium (K_{ATP}) channels couple energetic states with membrane excitability in neuronal and non-neuronal tissues. These channels associate tightly with glycolytic machinery (Dhar-Chowdhury et al., 2005, Ho et al., 2023), open when bound by Mg^{2+}-ADP, and are closed by high intracellular concentrations of ATP (Gribble et al., 1997, Tucker et al., 1998, Puljung et al., 2019). Thus, under low intracellular glucose concentrations, these channels open and reduce neuronal activity in hippocampal slices (Kawamura et al., 2010). Similarly, ketones inhibit glycolysis and open K_{ATP} channels, thereby reducing firing rates in slice and culture preparations from the central nervous system (Ma et al., 2007, Tanner et al., 2011, Lund et al., 2015). K_{ATP} channels regulate nociception, as they are expressed in the dorsal root ganglia (DRG) and dysregulated after peripheral nerve injury (Kawano et al., 2009, Luu et al., 2019). K_{ATP} channels also regulate opioid receptor signaling (Lohmann and Welch, 1999a, Lohmann and Welch, 1999b, Rodrigues and Duarte 2000), and their genetic elimination leads to mechanical allodynia and intraepidermal fiber loss characteristic of small-fiber neuropathy (Nakai-Shimoda et al., 2022). However, it is unclear whether ketone oxidation is associated with the activation of K_{ATP} channels in the somatosensory nervous system or whether a ketogenic diet requires K_{ATP} channel function to provide analgesia.

We tested the hypothesis that consumption of a ketogenic diet broadly provides antinociception by activating K_{ATP} channels. As an experimental model, we fed mice a ketogenic diet for one week before receiving a single intraplantar injection of various noxious stimuli—methylglyoxal, cinnamaldehyde (a TRPA1 agonist), capsaicin (a TRPV1 agonist), or Yoda1 (a PIEZO1 agonist)—and assessed nocifensive behaviors and markers of spinal neuron activity. We demonstrate that a ketogenic diet prevents pain behaviors in response to a range of noxious stimuli. Using a genetic mouse model of impaired ketone oxidation in peripheral sensory neurons and pharmacological inhibitors and activators of K_{ATP} channels, we demonstrate that this antinociception depends on neuronal ketone oxidation and K_{ATP} channel activity. These results identify a novel link between ketogenic diets, ketone oxidation, nociception, and K_{ATP} channel activation.

Materials and methods

Animals and diet

All animal work was performed following review and approval by the Institutional Animal Care and Use Committee of Kansas University Medical Center. Eight-week-old C57Bl/6 mice #027 were purchased from Charles River Laboratories (Willmington, MA). Sensory Neuron Advillin-Cre Knockout of *Oxct1* (Adv-KO-SCOT) mice were bred as previously described (Enders et al., 2023). All mice were maintained on a 12:12 light:dark cycle in the Kansas University Medical Center animal research facility. Mice were given *ad libitum* access to water and either a standard rodent chow (TD.8604; Envigo, Madison, WI; 14% fat, 32% protein, and 54% carbohydrate by kcal) or a ketogenic diet (TD.96355; Envigo, 90.5% fat, 9.2% protein, and 0.3% carbohydrate by kcal). Mice fed a ketogenic diet were provided a fresh diet every 3–4 days. All efforts were made to include an equal number of male and female mice in all studies (n = 6–8/group).

Noxious stimuli and drug administration

For peripheral administration in spontaneous nociception assays, methylglyoxal and cinnamaldehyde were diluted in sterile saline to working concentrations of 1.5 μg/μL (pH 7.0) and 0.65 μg/μL, respectively. Capsaicin was diluted in sterile saline with 0.5% Tween 20 to working concentrations of 0.2 μg/μL for spontaneous nociception assays and response to K_{ATP} channel blockade. Yoda1 (Tocris) was diluted in sterile saline with 5% dimethyl-sulfoxide (DMSO) to a working concentration of 355.27 ng/μL. Tolbutamide was diluted to a working concentration of 0.8 μg/μL in saline with 5% DMSO. For spontaneous nociception assay, methylglyoxal (30 μg), cinnamaldehyde (13 μg), capsaicin (4 μg), or Yoda1 (7.1054 μg) were delivered by a 20 μL subcutaneous injection to the right hind paw. Capsaicin (2 μg) and tolbutamide (8 μg) were delivered by subsequent 10 μL subcutaneous injections to the right hind paw to assess the contribution of K_{ATP} channels to antinociception by a ketogenic diet. Diazoxide (115 ng, Sigma) or levcromakalim (573 ng, MedChemExpress) were delivered in a 10 μL intraplantar injection 1 h before capsaicin (20 μg) to assess whether K_{ATP} channel activation could prevent capsaicin-evoked nociception. These doses were based on previously published work examining the effect of these drugs on nociception (Luu et al., 2019).

Sensory behaviors

The experimenter was blinded to all treatment groups during data collection, and animals were randomly assigned to treatment groups. Sensory behavioral testing was performed at baseline, 60-, and 90-minutes post-capsaicin injection for animals receiving intraplantar capsaicin and tolbutamide. Before collecting baseline data, mice were acclimated to testing areas for 30 min and either the mesh table for 30 min on at least two occasions, separated by 24 h. Before collecting mechanical threshold data, mice were again acclimated to the testing area and mesh table for 30 min each. Various Von Frey microfilaments (log1.65 to log 4.74) were applied to the plantar surface of the hind paw following the "up-down" method for one second. Animals were observed for either a negative or a positive response, and the mechanical withdrawal thresholds were calculated following five positive responses.

Sensory behavior in animals receiving intraplantar injections was determined by observation of spontaneous nocifensive behavior (e.g., licking, biting, lifting, and shaking the injected paw). Mice were acclimated to a clear plastic cage without bedding for 5 min before injection. Following intraplantar injection, mice were replaced in the cage. A blinded investigator then observed the mouse for 5 min following injection and recorded the total number of nocifensive events the animal displayed and the total time spent engaged in nocifensive behavior.

Spinal early activation in the dorsal horn

To assess the response of spinal dorsal horn cells to peripheral noxious stimulation, the lumbar enlargement of the spinal cord was dissected 10 min following intraplantar injection and post-fixed in 4% paraformaldehyde overnight. Spinal cords were cryopreserved in 30% sucrose, frozen in Optimal Cutting Temperature Compound (Sekura Tissue-Tek) and sectioned at 30 μm on a cryostat. Sections were blocked for two hours in Superblock (ThermoFisher; Grand Island, NY), 1.5% Normal Donkey Serum, 0.5% Porcine Gelatin, and 0.5% Triton X-100 (Sigma) at room temperature. Slides were incubated overnight at 4 °C with rabbit α-phospho-ERK 42/44 (1:500, Cell Signaling Technologies). Slides were next incubated with AlexaFluor-555 tagged donkey-α-rabbit secondary antibody (1:1000, Molecular Probes) for one hour and imaged with a Nikon Eclipse 90i or a Nikon Eclipse Ti2 inverted microscope. A blinded investigator counted the number of p-ERK^{+} cells in the superficial laminae of dorsal horn grey matter across three to five independent sections for each animal. To be counted, the cell had to 1) reside within the spinal dorsal horn, 2) be roughly spherical and

measure between 5 and 20 μm in diameter, and 3) display an increase in p-ERK fluorescence over background levels. The average count for each mouse was used for statistical analyses.

Statistical analyses

All statistical analyses were performed using R version 4.2.3 and packages "Rmisc", "car", "dplyr", and "ggpubr". All analyses for which data were collected over time were performed using a mixed-model analysis of variance (ANOVA) with repeated measures. All other analyses were performed using an N-way ANOVA. As appropriate, data were further analyzed *post hoc* by pairwise *t*-test or Tukey's Honest Significant Difference (HSD), as indicated. Shapiro-Wilks and Levene tests confirmed assumptions of normal distribution and homogeneity of variance, respectively. All data are presented as mean +/- standard error of the mean. All experiments were performed with male and female mice (n = 6–8/group) and analyzed for effect of sex. Sex differences are reported where present. Data were reanalyzed without sex as an independent variable where significant differences between sex and sex interactions were absent.

Results

A ketogenic diet mediates a broad analgesic effect

Mice were fed a ketogenic diet for one week before intraplantar injection of noxious stimuli (Fig. 1**A**). Mice injected with methylglyoxal demonstrated an increased number of nocifensive behaviors (Fig. 1**B** *left*; N-way ANOVA, methylglyoxal: $p < 2.73e^{-7}$) and increased time engaged in those behaviors (Fig. 1**B** *right*; N-way ANOVA, methylglyoxal: $p < 7.67e^{-7}$, diet-methylglyoxal interaction: $p < 0.0047$). Mice fed a ketogenic diet one week prior to methylglyoxal injection displayed significantly fewer nocifensive behaviors (*number of nocifensive events*, N-way ANOVA, diet: $p < 4.64e^{-6}$, diet-methylglyoxal interaction: $p < 2.48e^{-5}$; *time engaged in nocifensive behaviors*, N-way ANOVA, diet: $p < 0.00388$, diet-methylglyoxal interaction: $p < 0.0047$).

We postulated that the ketogenic diet-related antinociception in response to methylglyoxal-evoked nociception likely occurred too

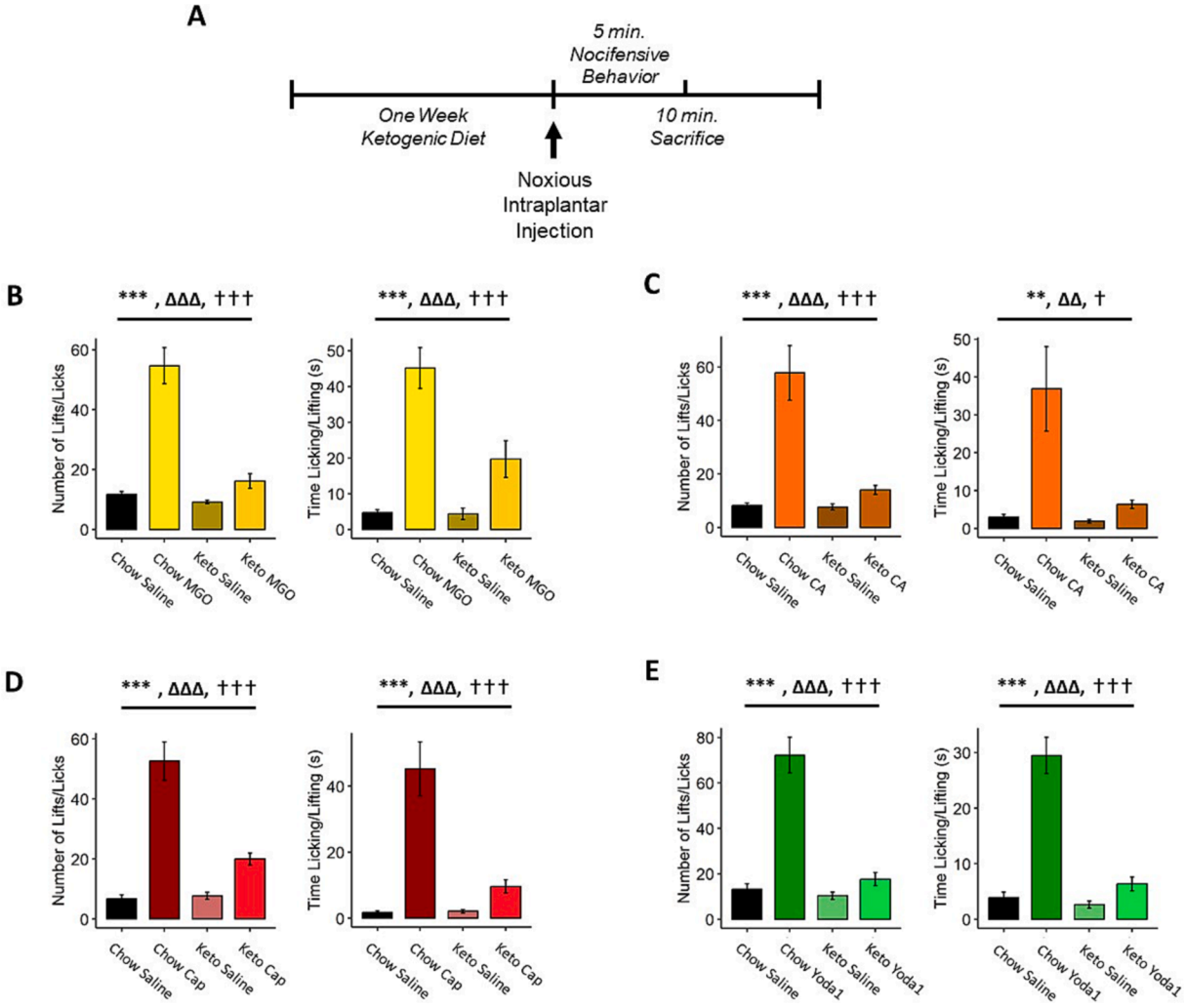


Fig. 1. A Ketogenic Diet Provides Antinociception to Diverse Noxious Stimuli. (*A*) The experimental design for determining the effect of a ketogenic diet on nociception. Mice were fed a ketogenic diet for one week before intraplantar injection of noxious stimuli. Mice were observed for nocifensive behavior (licking, biting, lifting, etc.) for five minutes following injection. Spinal cords were collected 10 min following the injection of the noxious stimulus. (*B*) Chow-fed mice injected with methylglyoxal (MGO) in more nocifensive behaviors (*left*) and spent more time engaged in those behaviors (*right*), whereas mice fed a ketogenic diet were protected. Increased nocifensive behaviors were also evoked by cinnamaldehyde (CA, *C*), capsaicin (Cap, *D*), and Yoda1 (*E*) in chow- but not ketogenic diet-fed mice. (*B-E*) N-way ANOVA (n = 7–8/group), ** denotes the effect of noxious stimulus: $p < 0.01$, *** denotes the effect of noxious stimulus: $p < 0.005$, ΔΔ denotes the effect of diet: $p < 0.01$, ΔΔΔ denotes the effect of diet: $p < 0.005$, + denotes the effect of stimulus-diet interaction: $p < 0.05$, +++ denotes the effect of stimulus-diet interaction: $p < 0.005$.

quickly to be explained by ketone bodies scavenging methylglyoxal (Salomón, Sibbersen et al. 2017). Methylglyoxal causes pain and pain-like behaviors in humans and rodents through direct activation of TRPA1 (Andersson et al., 2013, Griggs et al., 2017, Düll et al., 2019); thus, it is possible a ketogenic diet abrogates TRPA1-mediated nociception. To explore this possibility, we injected chow- and ketogenic diet-fed mice with cinnamaldehyde, a known TRPA1 agonist. Chow-fed mice injected with cinnamaldehyde displayed an increased number of nocifensive behaviors (Fig. 1**C** *left*; N-way ANOVA, cinnamaldehyde: $p < 1.05e^{-5}$) and engaged in nocifensive behaviors significantly longer than saline-injected mice (Fig. 1**C** *right*, N-way ANOVA, cinnamaldehyde: $p < 0.00199$). Again, mice fed a ketogenic diet were protected from cinnamaldehyde-induced nociception (*number of nocifensive events*, N-way ANOVA, diet: $p < 0.000225$, diet-cinnamaldehyde interaction: $p < 0.000292$; *time engaged in nocifensive behaviors*, N-way ANOVA, diet: $p < 0.00892$, diet-cinnamaldehyde interaction: $p < 0.01435$).

As both methylglyoxal and cinnamaldehyde signal through TRPA1, we next tested whether ketogenic diet-mediated antinociception was effective beyond TRPA1 regulation. To this end, we used the TRPV1 agonist capsaicin. Capsaicin increased the number of nocifensive behaviors (Fig. 1**D** *left*; N-way ANOVA, capsaicin: $p < 3.60e^{-9}$) and time engaged in those behaviors (Fig. 1**D** *right*; N-way ANOVA, capsaicin: $p < 1.55e^{-6}$). Capsaicin was unable to increase the number of nocifensive events or the time engaged in nocifensive behaviors in mice fed a ketogenic diet (*number of nocifensive events*, N-way ANOVA, diet: $p < 8.55e^{-5}$, diet-capsaicin interaction: $p < 3.87e^{-5}$; *time engaged in nocifensive behaviors*, N-way ANOVA, diet: $p < 0.000252$, diet-capsaicin interaction: $p < 0.000198$). Together, these data suggest an anti-nociceptive activity of ketogenic diets that is not specific to a single TRP channel.

TRPA1 and TRPV1 channels physically interact with each other, and during this interaction activity of these channels regulate each other (Staruschenko et al., 2010, Weng et al., 2015). To eliminate the possibility that the antinociceptive effect of a ketogenic diet depends on the regulation of a TRPA1-TRPV1 complex, we assessed nociceptive responses to Yoda1, a chemical activator of the mechanosensitive channel PIEZO1, following administration of a ketogenic diet. Intraplantar Yoda1 evoked a significant increase in the number of nocifensive behaviors (Fig. 1**E**, *left*; N-way ANOVA, Yoda1: $p < 4.03e^{-08}$) and the amount of time engaged in such behaviors (Fig. 1**E**, *right*; N-way ANOVA, Yoda1: $p < 1.17e^{-8}$). These behaviors were prevented in mice fed a ketogenic diet (*number of nocifensive events*, N-way ANOVA, diet: $p < 5.19e^{-7}$, diet-Yoda1 interaction: $p < 2.94^{-6}$; *time engaged in nocifensive behaviors*, N-way ANOVA, diet: $p < 3.73e^{-7}$, diet-Yoda1 interaction: $p <$

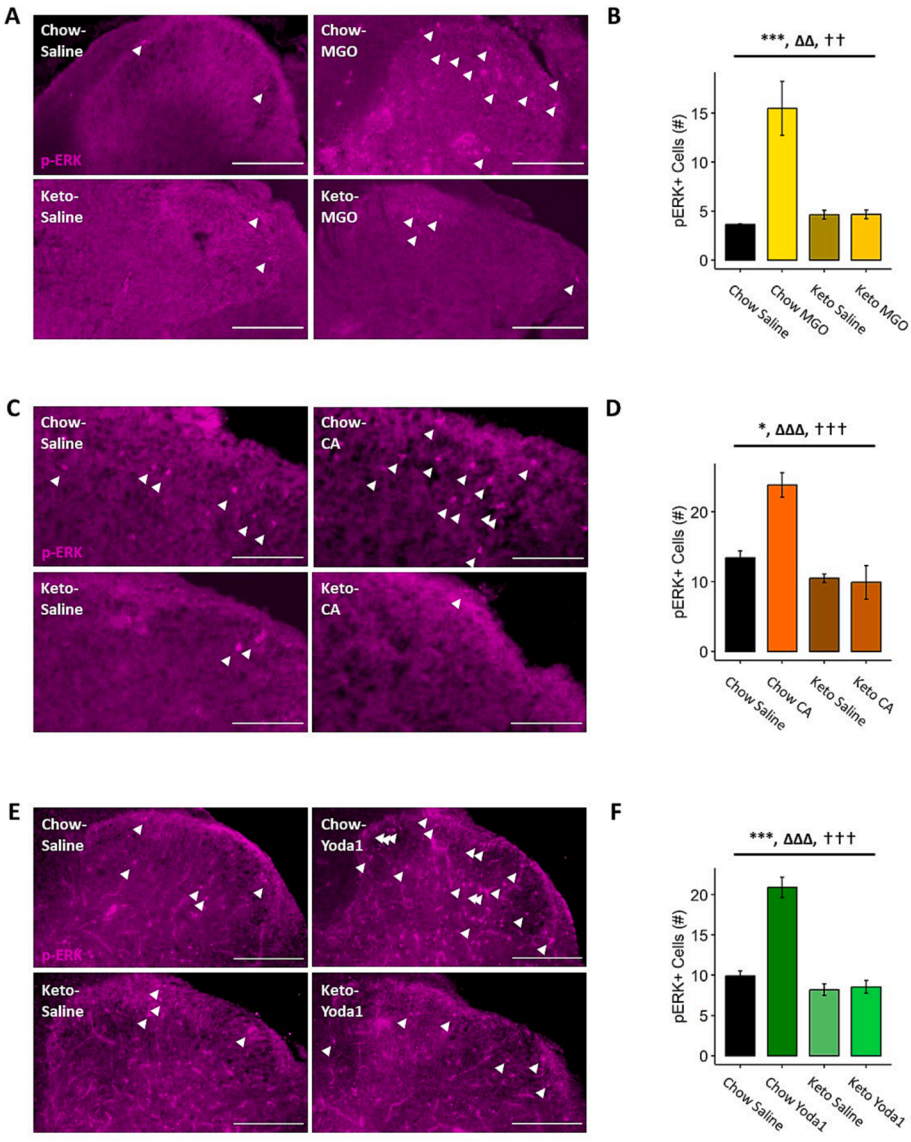


Fig. 2. A Ketogenic Diet Prevents Noxious Stimulus-Evoked Early Activation in the Spinal Dorsal Horn. (*A-B*) Methylglyoxal increases the number of phospho-ERK (p-ERK$^+$, *white arrowheads*) cells in the spinal dorsal horn within 10 min of intraplantar injection in chow-fed mice. Mice fed a ketogenic diet are protected from methylglyoxal-evoked early activation. (*C-D*) Cinnamaldehyde increases p-ERK$^+$ cell number in the spinal dorsal horn in chow-fed mice but not mice fed a ketogenic diet. (*E-F*) Yoda1 increases p-ERK$^+$ cell counts in the spinal dorsal horn of chow-fed mice within 10 min of injection. Mice fed a ketogenic diet are protected from Yoda1-mediated p-ERK$^+$ cell number increase. (*B, D, F*) N-way ANOVA (n = 3–4/group), * denotes the effect of noxious stimulus: $p < 0.05$, *** denotes the effect of noxious stimulus: $p < 0.005$, ΔΔ denotes the effect of diet: $p < 0.01$, ΔΔΔ denotes the effect of diet: $p < 0.005$, ++ denotes the effect of stimulus-diet interaction: $p < 0.01$, +++ denotes the effect of stimulus-diet interaction: $p < 0.005$. The scale bar represents 200 μm.

$2.27e^{-6}$), indicating that a ketogenic diet also reduces nociception mediated by PIEZO1 activity.

A ketogenic diet decreases early activation markers in the spinal dorsal horn following peripheral noxious stimuli

To measure whether a ketogenic diet affects the transmission of noxious stimuli to spinal neurons, we quantified the number of phosphorylated extracellular signal-related kinase (p-ERK) positive cells in the spinal dorsal horn ipsilateral to the intraplantar injection of a noxious stimulus. Consistent with our behavioral data, methylglyoxal increased the number of p-ERK$^+$ cells in the spinal dorsal horn of chow-fed mice (Fig. 2**A-B**; N-way ANOVA, methylglyoxal: $p < 0.00294$). Consumption of a ketogenic diet for one week prevented this increase in p-ERK$^+$ cell number (N-way ANOVA, diet: $p < 0.00826$, diet-methylglyoxal interaction: $p < 0.00306$). Cinnamaldehyde increased the number of p-ERK$^+$ cells in chow-fed but not ketogenic diet-fed mice (Fig. 2**C-D**; N-way ANOVA, cinnamaldehyde: $p < 0.02124$, diet: $p < 9.33e^{-5}$, diet-cinnamaldehyde interaction: $p < 0.00499$). A ketogenic diet also prevented Yoda1-dependent increases in p-ERK$^+$ cell number in the spinal dorsal horn (Fig. 2**E-F**; N-way ANOVA, Yoda1: $p < 3.6e^{-5}$, diet: $p < 4.41e^{-6}$, diet-Yoda1 interaction: $p < 6.51e^{-5}$). These findings suggest that consumption of a ketogenic diet inhibits neuronal activation in response to noxious stimuli in the spinal dorsal horn.

Ketone oxidation is required for the analgesic effect of a ketogenic diet

We next asked whether ketone oxidation as fuel in peripheral neurons was necessary to mediate the analgesic effect of a ketogenic diet. We followed the same experimental design (Fig. 1**A**) but used sensory neuron-specific Advillin-Cre knockout of *Oxct1* (Adv-KO-SCOT) mice. These mice have a tissue-specific knockout of succinyl-CoA 3-oxoacid CoA-transferase 1 (SCOT, encoded by *Oxct1*) and cannot oxidize ketone bodies for fuel in peripheral sensory neurons. As before, methylglyoxal increased the number of nociceptive events (Fig. 3**A**; N-way ANOVA, methylglyoxal: $p < 9.26e^{-12}$) and the amount of time engaged in nocifensive behavior (Fig. 3**B**; N-way ANOVA, methylglyoxal: $p < 6.83e^{-8}$) in both Adv-KO-SCOT and littermate wildtype-control mice. We also detected significant effects of genotype (*number of nocifensive events*, $p < 0.033$; *time engaged in nocifensive behaviors*, $p < 0.01166$) and genotype-methylglyoxal interaction (*number of nocifensive events*, $p < 0.00739$; *time engaged in nocifensive behaviors*, $p < 0.00627$), indicating that Adv-KO-SCOT mice may be more susceptible to methylglyoxal-evoked nociception regardless of diet. We also detected a significant effect of consuming a ketogenic diet and the interaction between consuming a ketogenic diet and methylglyoxal injection on both the number of nocifensive responses (Fig. 3**A**; N-way ANOVA, diet: $p < 3.34e^{-5}$, diet-methylglyoxal interaction: $p < 2.41e^{-7}$) and the time engaged in those responses (Fig. 3**B**; N-way ANOVA, diet: $p < 0.00241$, diet-methylglyoxal interaction: $p < 5.17e^{-6}$), indicating a ketone oxidation-independent analgesic effect of a ketogenic diet.

Prior to data collection, we compared the responses of wildtype and Adv-KO-SCOT ketogenic diet-fed, methylglyoxal-injected mice. This analysis revealed a significant increase in the number of nocifensive responses (Fig. 3**A**, planned comparison by Student's *t*-test, $p < 0.002692$) and the time engaged in nocifensive behaviors (Fig. 3**B**, planned comparison by Student's *t*-test, $p < 0.02995$) in Adv-KO-SCOT compared to wildtype ketogenic diet-fed, methylglyoxal-injected mice. These results support a mechanism by which ketone oxidation mediates, in part, some of the protective effects of a ketogenic diet against methylglyoxal-evoked nociception.

A ketogenic diet is unable to reduce p-ERK expression in the spinal dorsal horn in mice lacking the ability to oxidize ketones in peripheral neurons

We assessed p-ERK expression in the spinal dorsal horn of ketogenic diet-fed Adv-KO-SCOT mice following methylglyoxal injection. Methylglyoxal increased the number of p-ERK$^+$ cells in the spinal dorsal horn of chow-fed wildtype and Adv-KO-SCOT mice (Fig. 4; N-way ANOVA, methylglyoxal: $p < 8.74e^{-9}$). We detected a significant interaction between the ketogenic diet and methylglyoxal (N-way ANOVA, ketogenic diet-methylglyoxal interaction: $p < 1.78e^{-6}$), indicating a ketone oxidation-independent effect on the number of spinal p-ERK$^+$ cells following methylglyoxal injection. Prior to data analysis, we set planned comparisons *a priori* or spinal p-ERK$^+$ expression between wildtype and Adv-KO-SCOT ketogenic diet-fed, methylglyoxal-injected mice. Adv-KO-SCOT ketogenic diet-fed, methylglyoxal-injected mice had significantly more p-ERK$^+$ cells compared to wildtype mice (Fig. 4, planned comparison by Student's *t*-test: $p < 0.000501$), correlating with nociceptive behavior responses and suggesting that peripheral sensory neuron ketone oxidation is required for a ketogenic diet to reduce methylglyoxal-evoked spinal dorsal horn activity.

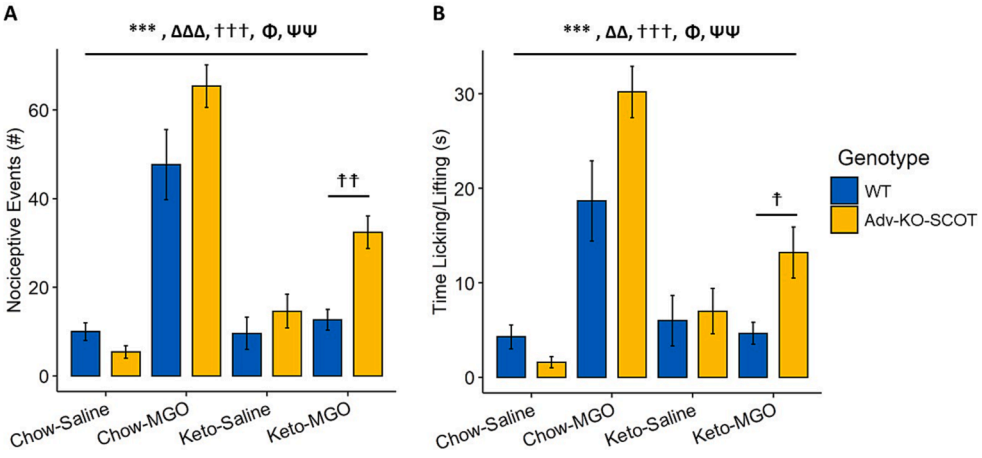


Fig. 3. Ketone Oxidation is Required for the Full Antinociceptive Effect of a Ketogenic Diet. Wildtype (WT) and sensory neuron-specific *Advillin*-Cre knockout of *Oxct1* (Adv-KO-SCOT) mice were fed a ketogenic diet for one week before intraplantar methylglyoxal injection. Methylglyoxal increased the number of nocifensive events (*A*) and the time engaged nocifensive behaviors (*B*) in both WT and Adv-KO-SCOT mice. Consumption of a ketogenic diet reduced methylglyoxal-evoked nociception in both WT and Adv-KO-SCOT mice, though ketogenic diet-fed, methylglyoxal-injected Adv-KO-SCOT mice exhibited more nociceptive events (*A*) and engaged in nocifensive behaviors (*B*) longer than ketogenic diet-fed, methylglyoxal-injected WT mice. (*A-B*) N-way ANOVA (n = 6–9/group), *** denotes the effect of noxious stimulus: $p < 0.005$, ΔΔ denotes the effect of diet: $p < 0.01$, ΔΔΔ denotes the effect of diet: $p < 0.005$, ++ denotes the effect of stimulus-diet interaction: $p < 0.01$, +++ denotes the effect of stimulus-diet interactio+: $p < 0.005$, Φ denotes the effect of genotype: $p < 0.05$, ΨΨ denotes the effect of genotype-stimulus interaction: $p < 0.01$. *A priori* planned comparisons, Student's *t*-test denotes the difference between WT and Adv-KO-SCOT ketogenic diet-fed, methylglyoxal injected mice: $p < 0.05$, denotes the difference between WT and Adv-KO-SCOT ketogenic diet-fed, methylglyoxal injected mice: $p < 0.01$.

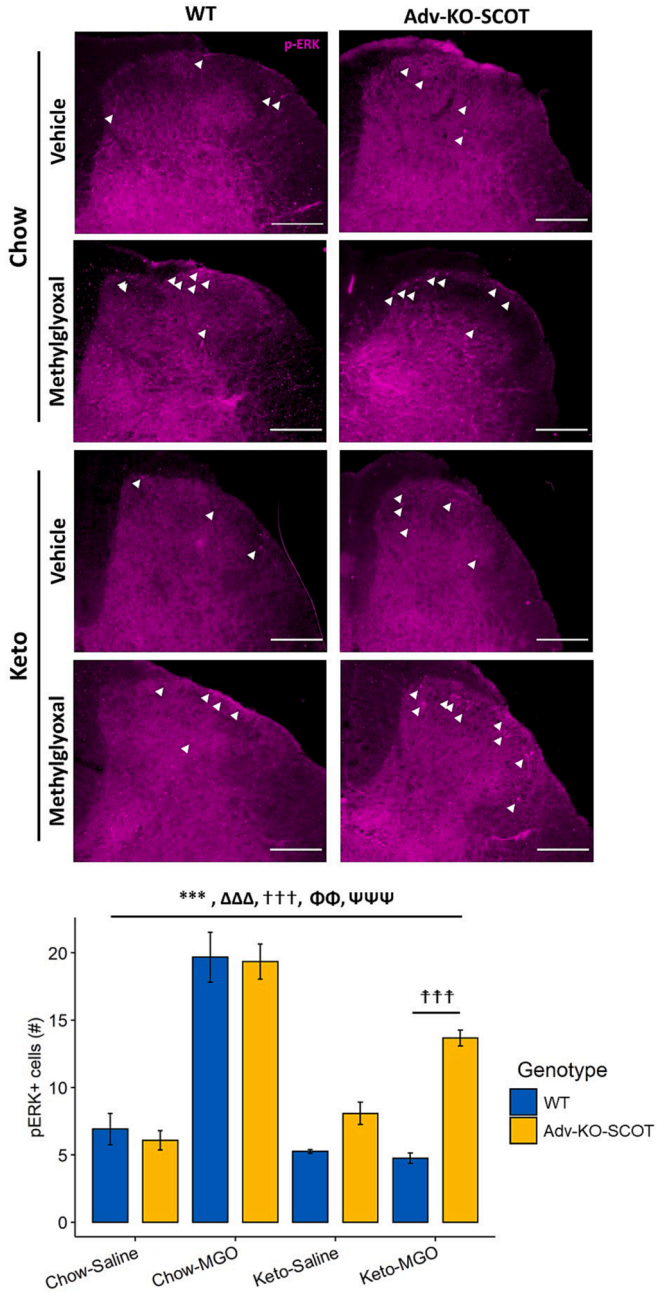


Fig. 4. Ketone Oxidation is Required for a Ketogenic Diet to Reduce p-ERK Expression in the Spinal Dorsal Horn Following Noxious Stimuli. Intraplantar methylglyoxal injection increased the number of p-ERK$^+$ cells (*white arrowheads*) in the spinal dorsal of both WT and Adv-KO-SCOT mice. Consumption of a ketogenic diet one week before methylglyoxal injection reduced the number of p-ERK$^+$ cells in both genotypes; however, ketogenic diet-fed, methylglyoxal-injected Adv-KO-SCOT mice had more p-ERK$^+$ cells in the spinal dorsal horn than ketogenic diet-fed, methylglyoxal-injected WT mice. N-way ANOVA (n = 3/group), *** denotes + he effect of noxious stimulus: $p < 0.005$, ΔΔΔ denotes the effect of diet: $p < 0.005$, +++ denotes the effect of stimulus-diet interaction: $p < 0.005$, ΦΦ denotes the effect of genotype: $p < 0.01$, ΨΨΨ denotes the effect of genotype-stimulus interaction: $p < 0.005$. *A priori* planned comparisons, Student's *t*-test, denotes the difference between WT and Adv-KO-SCOT ketogenic diet-fed, methylglyoxal injected mice: $p < 0.005$. The scale bar represents 200 μm.

ATP-gated potassium (K_{ATP}) channels are required for ketogenic diet-mediated Analgesia

To test whether the analgesic activity of a ketogenic diet requires K_{ATP} channels, we assessed the mechanical thresholds of ketogenic diet-fed mice receiving subsequent intraplantar injections of capsaicin and tolbutamide, a K_{ATP} channel antagonist. One hour after capsaicin injection, chow-fed mice appropriately developed mechanical allodynia, while ketogenic diet-fed mice were protected (Fig. 5**A**; N-way mixed-models ANOVA with repeated measures, diet-capsaicin-time interaction: $p < 0.00421$; Tukey's *post hoc* test, chow-before capsaicin-before tolbutamide compared to chow-after capsaicin-before tolbutamide: adjusted $p < 1.00e^{-7}$, ketogenic diet-before capsaicin-before tolbutamide compared to ketogenic diet-after capsaicin-before tolbutamide: adjusted $p < 1.0$). In vehicle-injected mice, tolbutamide did not cause mechanical allodynia regardless of the diet consumed (Tukey's *post hoc*, vehicle-before tolbutamide compared to vehicle-after tolbutamide: adjusted $p < 0.999$). Within 30 min of tolbutamide injection, however, ketogenic diet-fed, capsaicin-injected mice quickly developed mechanical allodynia similar to chow-fed capsaicin-injected mice (Tukey's *post hoc*, ketogenic diet-after capsaicin-before tolbutamide compared to ketogenic diet-after capsaicin-after tolbutamide: $p < 1.00e^{-7}$, chow-after capsaicin-before tolbutamide compared to ketogenic diet-after capsaicin-after tolbutamide: $p < 1.00$).

In a separate experiment, we fed mice a ketogenic diet for one week, and tolbutamide was injected intraplantar 30 min before capsaicin. Consistent with our previous result, capsaicin increased the number of nocifensive behaviors (Fig. 5**B**; N-way ANOVA, diet: $p < 0.01107$, capsaicin: $p < 2e^{-16}$, diet-capsaicin interaction: $p < 0.04189$) and time engaged in nocifensive behaviors (Fig. 5**C**; N-way ANOVA, diet: $p < 0.00563$, capsaicin: $p < 2e^{-16}$, diet-capsaicin interaction: $p < 0.0057$) in chow- but not ketogenic diet-fed mice. These behavioral responses were prevented by prior injection with tolbutamide (*number of nocifensive events*, N-way ANOVA, diet-tolbutamide-capsaicin interaction: $p < 0.035$; *time engaged in nocifensive behaviors*, N-way ANOVA, diet-tolbutamide-capsaicin interaction: $p < 0.0166$), suggesting that antagonism of K_{ATP} channel activity can override the analgesic effect of a ketogenic diet.

K_{ATP} channels are required for ketogenic diet-mediated reduction of p-ERK in the spinal dorsal horn

We quantified p-ERK$^+$ cells in the spinal dorsal horn in mice fed a ketogenic diet following tolbutamide injection. Mice receiving capsaicin had a significant increase in the number of p-ERK$^+$ cells in the spinal dorsal horn compared to vehicle-injected mice (Fig. 6; N-way ANOVA, capsaicin: $p < 1.24e^{-13}$). This increase was prevented in mice fed a ketogenic diet (N-way ANOVA, diet: $p < 1.15e^{-9}$, diet-capsaicin interaction: $p < 4.57e^{-9}$). The ketogenic diet-mediated reduction in spinal p-ERK$^+$ cells following capsaicin injection was prevented, however, by injection of tolbutamide (N-way ANOVA, diet-tolbutamide-capsaicin interaction: $p < 0.000107$). This result suggests that a ketogenic diet requires K_{ATP} channel activity to prevent peripheral noxious stimuli from reaching the spinal dorsal horn.

Peripheral activation of SUR1-containing K_{ATP} channels mediates analgesic effect of a ketogenic diet

To determine whether activation of K_{ATP} channels was sufficient to recapitulate antinociception provided by a ketogenic diet, we injected chow-fed mice with diazoxide, an activator of K_{ATP} channels, one hour before intraplantar capsaicin injection. Mice injected with diazoxide before receiving capsaicin were protected from capsaicin-evoked nocifensive events (Fig. 7**A**, N-way ANOVA, capsaicin: $p < 1.14e^{-8}$, diazoxide: $p < 0.000217$, capsaicin-diazoxide interaction: $p < 0.000156$) and spent less time engaged in nocifensive behaviors (Fig. 7**B**, N-way

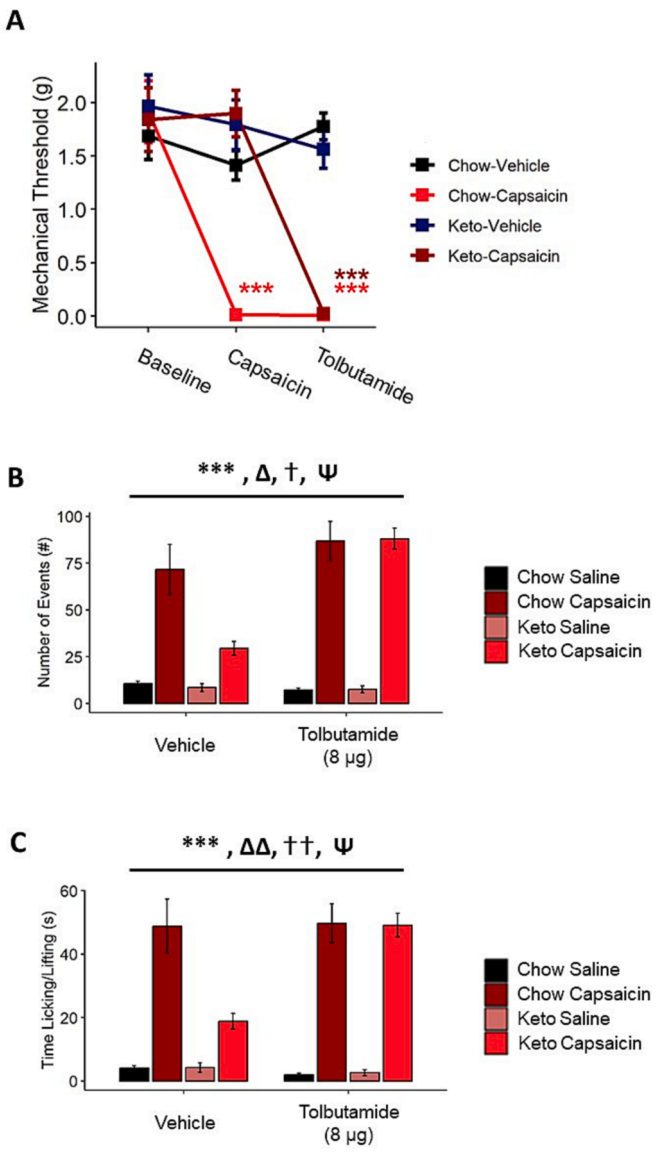


Fig. 5. K_{ATP} Channel Activity is Required for a Ketogenic Diet to Provide an Antinociception. (*A*) Capsaicin caused mechanical allodynia in chow-fed mice one-hour following intraplantar injection. Mice fed a ketogenic diet one week before injection were protected from capsaicin-evoked mechanical allodynia. 30 min following ipsilateral intraplantar injection of tolbutamide, ketogenic diet-fed, capsaicin-injected mice developed mechanical allodynia. Neither chow- nor ketogenic diet-fed mice developed mechanical allodynia following tolbutamide without capsaicin. Capsaicin caused increased nociceptive events (*B*) and increased time engaged in nocifensive behaviors (*C*) in chow-fed mice but not mice fed a ketogenic diet one week before injection. Intraplantar injection of tolbutamide 30 min before capsaicin injection prevented protection from capsaicin-evoked nociception in ketogenic diet-fed mice. (*A*) N-way, mixed models ANOVA with Tukey's *post hoc* test (n = 7–8/group), *** indicates comparison to chow-fed, vehicle-injected: $p < 0.005$, color indicates the group. (*B-C*) N-way ANOVA (n = 7–8/group), *** denotes the effect of noxious stimulus: $p < 0.005$, Δ denotes the effect of diet: $p < 0.05$, + denotes the effect of stimulus-diet interaction: $p < 0.05$, Ψ denotes the effect of stimulus-diet-tolbutamide interaction: $p < 0.05$.

ANOVA, capsaicin: $< 4.24e^{-8}$, diazoxide: $p < 0.000203$, capsaicin-diazoxide interaction: $p < 0.000203$) compared to vehicle-injected mice.

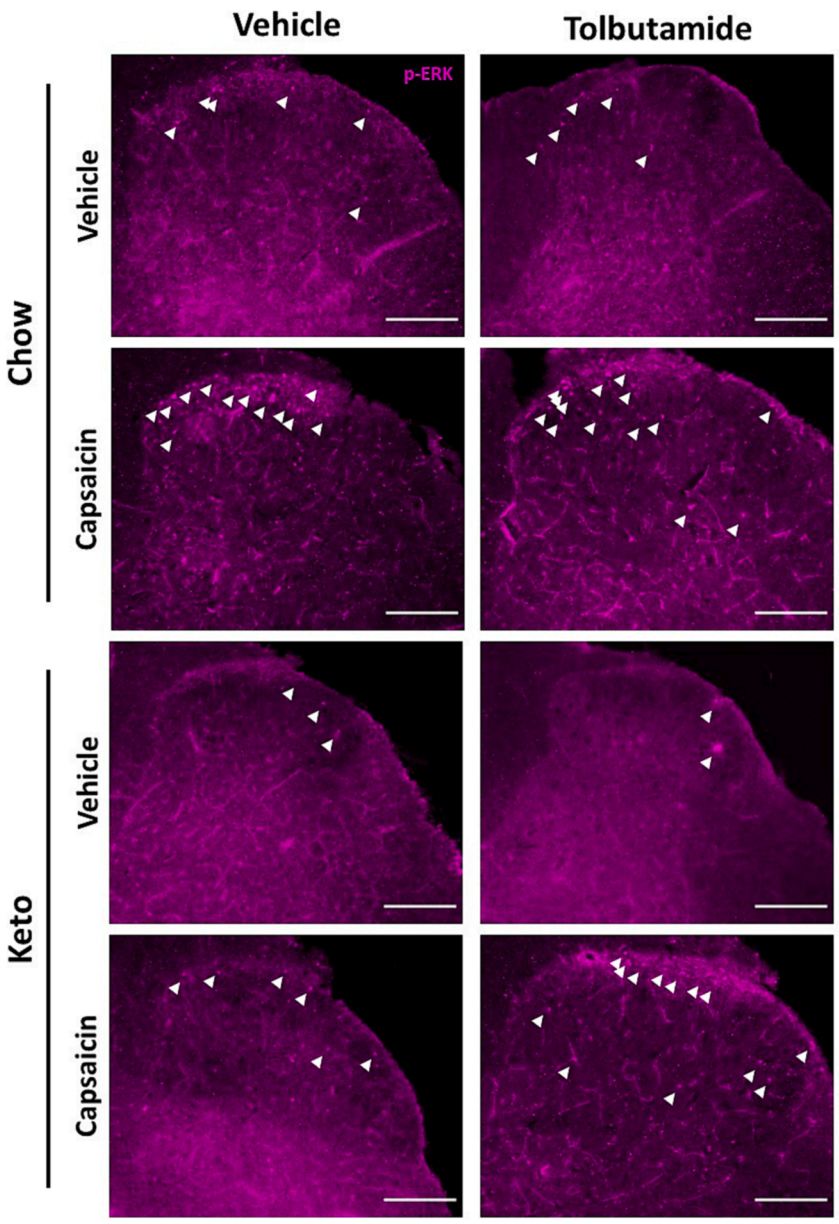
While diazoxide is selective for K_{ATP} channels containing the sulfonylurea receptor 1 (SUR1) regulatory subunit, SUR2B-containing K_{ATP} channels retain some sensitivity to diazoxide (Inagaki et al., 1996, Matsuoka et al., 2000, Wheeler et al., 2008). Levcromakalim, however, selectively activates K_{ATP} channels containing SUR2A and SUR2B, but not SUR1 (Schwanstecher et al., 1998). To determine whether there was selectivity between K_{ATP} channel subunit composition and antinociception, we injected mice with levcromakalim one hour before intraplantar capsaicin injection. Mice injected with levcromakalim before capsaicin showed only modest protection from the number of capsaicin-evoked nocifensive behaviors (Fig. 7**C**, N-way ANOVA capsaicin: $p < 5.35e^{-13}$, levcromakalim: $p < 0.688$, capsaicin-levcromakalim interaction: $p < 0.0251$). Levcromakalim also modestly reduced the time engaged in capsaicin-evoked nocifensive behaviors (Fig. 7**D**, N-way ANOVA capsaicin: $p < 9.21e^{-11}$, levcromakalim: $p < 0.397$, capsaicin-levcromakalim interaction: $p < 0.0388$). While we detected a significant interaction between levcromakalim and capsaicin injection for both the number of nocifensive events and the amount of time engaged in nocifensive behavior, these behaviors were not significantly different between mice injected with vehicle and capsaicin and those injected with levcromakalim and capsaicin (number of nocifensive events: Tukey's *post hoc*: $p = 0.127$; time engaged in nocifensive behaviors: Tukey's *post hoc*: $p = 0.102$).

While capsaicin increased the number of p-ERK^+ cells in the spinal dorsal horn of vehicle-injected mice (Fig. 8**A-B**, N-way ANOVA, capsaicin: $p < 4.95e^{-5}$), diazoxide significantly reduced the number of p-ERK^+ cells (N-way ANOVA, diazoxide: $p < 0.000314$, capsaicin-diazoxide interaction: $p < 0.003498$). Prior injection with levcromakalim did not prevent increased p-ERK^+ cell count in the spinal dorsal horn following capsaicin injection (Fig. 8**C-D**; N-way ANOVA, capsaicin: $p < 5.72e^{-7}$, levcromakalim: $p = 0.569$, capsaicin-levcromakalim interaction: $p < 0.00684$).

Discussion

There is mounting evidence that consuming a ketogenic diet is protective against pain and pain-like behaviors in clinical and preclinical settings. Our group has previously reported that consumption of a ketogenic diet prevents and reverses mechanical allodynia in mouse models of obesity (Cooper et al., 2018b) and diabetic peripheral neuropathy (Enders et al., 2022a). Others have demonstrated that ketogenic diets reduce pain-like behaviors in rodent models of inflammatory pain (Ruskin et al., 2009, Ruskin et al., 2021) and chemotherapy-induced neuropathy (Zhong et al., 2021) and reduce pain in patients with migraine (Di Lorenzo et al., 2019, Bongiovanni et al., 2021) and chronic musculoskeletal pain (Field et al., 2022). However, the mechanisms underlying this analgesia remain poorly defined. Possible contributions include improved metabolic neuronal health, as mice fed a ketogenic diet produce fewer reactive oxygen species in their sciatic nerve (Cooper et al., 2018a), and a ketogenic diet and ketone availability contribute to methylglyoxal detoxification (Salomón et al., 2017, Enders et al., 2022b), It is plausible that this mechanism is important in reducing pain-like behaviors in experimental models of diabetic neuropathy (Zherebitskaya et al., 2009, Bierhaus et al., 2012, Eberhardt et al., 2012, Rojas et al., 2018, Griggs et al., 2019).

In this study, we demonstrate that a ketogenic diet is broadly antinociceptive to acute administration of several different noxious stimuli, including capsaicin, cinnamaldehyde, methylglyoxal, and Yoda1. As expected, intraplantar injection of these noxious agents induced nocifensive behaviors (licking, lifting, biting, etc. of the injected paw) in mice. These behaviors were diminished in mice fed a ketogenic diet for all these stimuli (Fig. 1). Methylglyoxal-evoked nociception has been best-studied through activation of TRPA1 (Eberhardt et al., 2012, Andersson et al., 2013, Griggs et al., 2017, Düll et al., 2019); however, inhibition of TRPA1 does not fully prevent nociception after methylglyoxal (Barragán-Iglesias et al., 2019), and methylglyoxal promotes pain-like behaviors through TRPA1-independent mechanisms that include glycation of $Na_V1.8$ (Bierhaus et al., 2012), activation of the receptor for advanced glycation end products (Wei et al., 2017), and



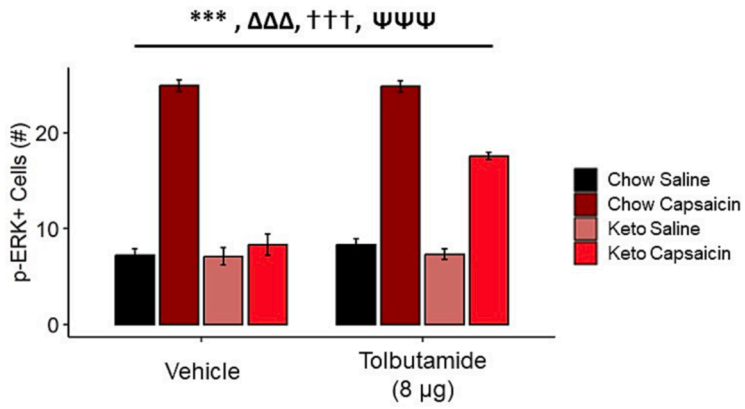


Fig. 6. K_{ATP} Channels Are Required to Reduce p-ERK Expression Following Capsaicin Injection in Mice Fed a Ketogenic Diet. Intraplantar capsaicin injection increased the number of p-ERK$^+$ cells (*white arrowheads*) in the spinal dorsal horn of chow-fed mice. Mice fed a ketogenic diet before capsaicin injection were protected from this increase in p-ERK$^+$ cells. Intraplantar injection of tolbutamide 30 min before capsaicin injection increased the number of p-ERK$^+$ cells in the spinal dorsal horn of ketogenic diet-fed mice. Tolbutamide injection did not affect the number of p-ERK$^+$ cells in chow-fed capsaicin-injected mice. N-way ANOVA ($n = 3$–4/group), *** denotes the effect of noxious stimulus: $p < 0.005$, ΔΔΔ denotes the effect of diet: $p < 0.005$, +++ denotes the effect of stimulus-diet interaction: $p < 0.005$, ΨΨΨ denotes the effect of stimulus-diet-tolbutamide interaction: $p < 0.005$.

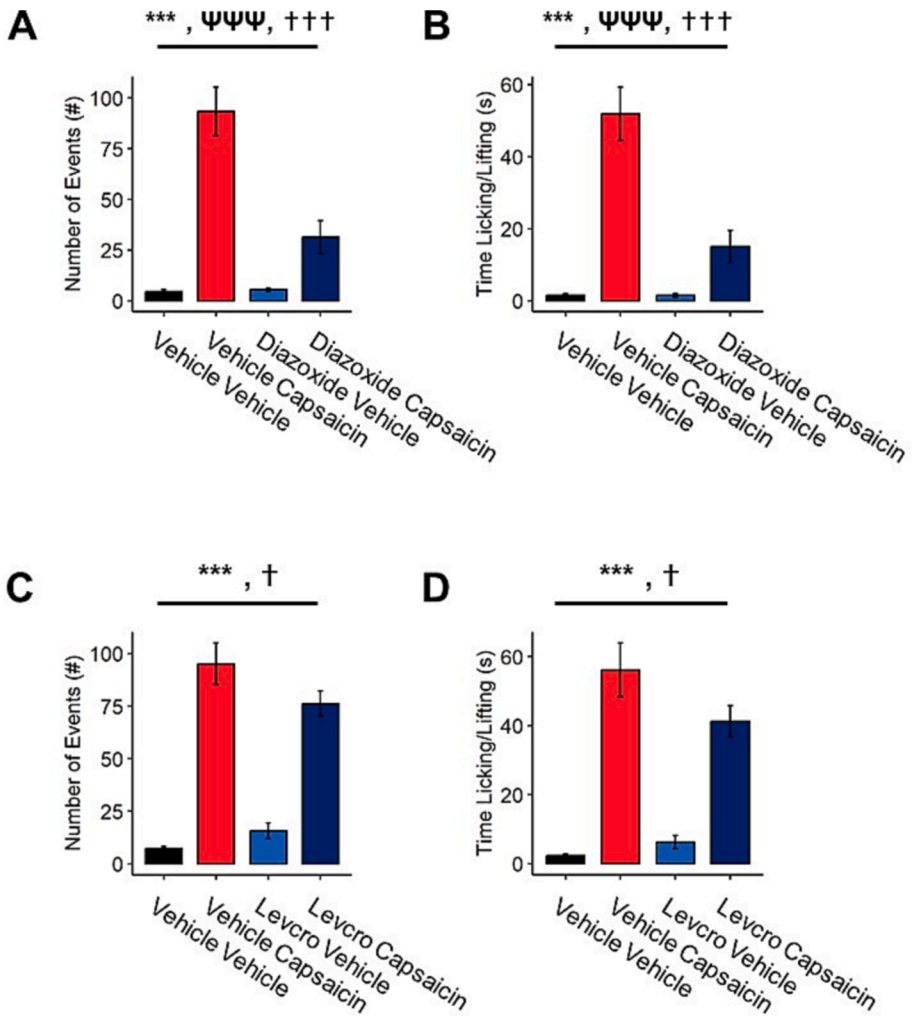


Fig. 7. Activation of SUR1-Containing K_{ATP} Channels Mimics the Antinociceptive Effect of a Ketogenic Diet. Capsaicin increased nociceptive events (*A, C*) and increased time engaged in nocifensive behavior (*B, D*) in chow-fed mice. Intraplantar injection of diazoxide one hour before capsaicin injection was sufficient to rescue capsaicin-evoked nociception (*A-B*), while intraplantar injection of levcromakalim (levcro) offered only modest protection (*C-D*). (*A-D*) N-way ANOVA (n = 8/group), *** denotes the effect of noxious stimulus: $p < 0.005$, ΨΨΨ denotes the effect of K_{ATP} channel openers: $p < 0.005$, + denotes the effect of stimulus-K_{ATP} openers interaction: $p < 0.05$, +++the effect of stimulus-K_{ATP} openers interaction: $p < 0.005$.

activation of the integrated stress response (Barragán-Iglesias et al., 2019). Thus, the ability of a ketogenic diet to reduce pain-like behaviors in response to cinnamaldehyde (TRPA1 agonist), capsaicin (TRPV1 agonist), and methylglyoxal suggests the antinociceptive mechanism of a ketogenic diet is not restricted to methylglyoxal detoxification (Enders et al., 2022b).

TRPA1 and TRPV1 physically interact and modify each other's activity (Staruschenko et al., 2010, Weng et al., 2015). Thus, one potential antinociceptive mechanism of a ketogenic diet is regulation of the TRPA1-TRPV1 macromolecular complex. While data here do not eliminate this possibility, we demonstrate that consumption of a ketogenic diet prevents nociceptive behaviors in response to Yoda1, a chemical activator of PIEZO1. PIEZO1 is a mechanosensitive channel expressed by neuronal and non-neuronal cells (Coste et al., 2010, Lee et al., 2014, Wang et al., 2019, Mikesell et al., 2022). Due to the differential expression of TRPV1, TRPA1, and PIEZO1, it is unlikely that PIEZO1 activity is regulated by these other channels (Wang et al., 2019). Since consumption of a ketogenic diet prevents PIEZO1-mediated nociception, we believe that a ketogenic diet provides a broader mechanism of antinociception than modifying these ion channels individually.

As such, we focused on the possibility of reducing the membrane excitability of nociceptors. In slice and dissociated culture recordings from the central nervous system, ketones can reduce spontaneous firing rates associated with reduced glycolytic flux and activation of K_{ATP} channels (Ma et al., 2007, Tanner et al., 2011, Lund et al., 2015). K_{ATP} channels are inhibited by ATP (Gribble et al., 1997, Tucker et al., 1998, Puljung et al., 2019) and associate closely with cellular glycolytic machinery (Dhar-Chowdhury, Harrell et al. 2005, Ho et al., 2023). Others have suggested that glycolysis increases ATP concentrations near the membrane, leading to the closure of K_{ATP} channels (Fig. 9**A**). Ketones inhibit glycolysis (Ma et al., 2007, Lund et al., 2015, Valdebenito et al., 2015), shifting the cell toward oxidative metabolism in the mitochondria and diminishing the membrane-proximal ATP pool. This combination results in the opening of K_{ATP} channels and hyperpolarization of the cell (Fig. 9**B**).

Relevant to pain, dysregulation of K_{ATP} channels has been reported in rodent models of neuropathy. Upregulation of K_{ATP} channel subunits occurs following spinal nerve ligation (Luu et al., 2019) and in a model of diabetic neuropathy (Nakai-Shimoda et al., 2022). This may represent a compensatory mechanism, as knockout of the Kir6.2 or SUR1 subunits results in mechanical and thermal hyperalgesia (Luu et al., 2019, Nakai-Shimoda et al., 2022) and spinal nerve ligation reduced K_{ATP} conductance in the DRG of mice following spinal nerve ligation (Kawano et al., 2009). Glibenclamide, another sulfonylurea antagonist of K_{ATP} channels, increases the resting membrane potential of uninjured DRG sensory neurons (Kawano et al., 2009). Conversely, opening K_{ATP} channels with the agonist diazoxide reduces C-fiber excitability in response to mechanical stimuli (Luu et al., 2019).

Collectively, these results suggest a mechanism in which K_{ATP} channel activity hyperpolarizes neurons to reduce their firing, consistent with the results presented here. As in previous studies, we show that mice fed a ketogenic diet were protected from methylglyoxal-evoked nociception (Enders et al., 2022b). It is important to point out that Adv-KO-SCOT mice were not fully protected from methylglyoxal-evoked

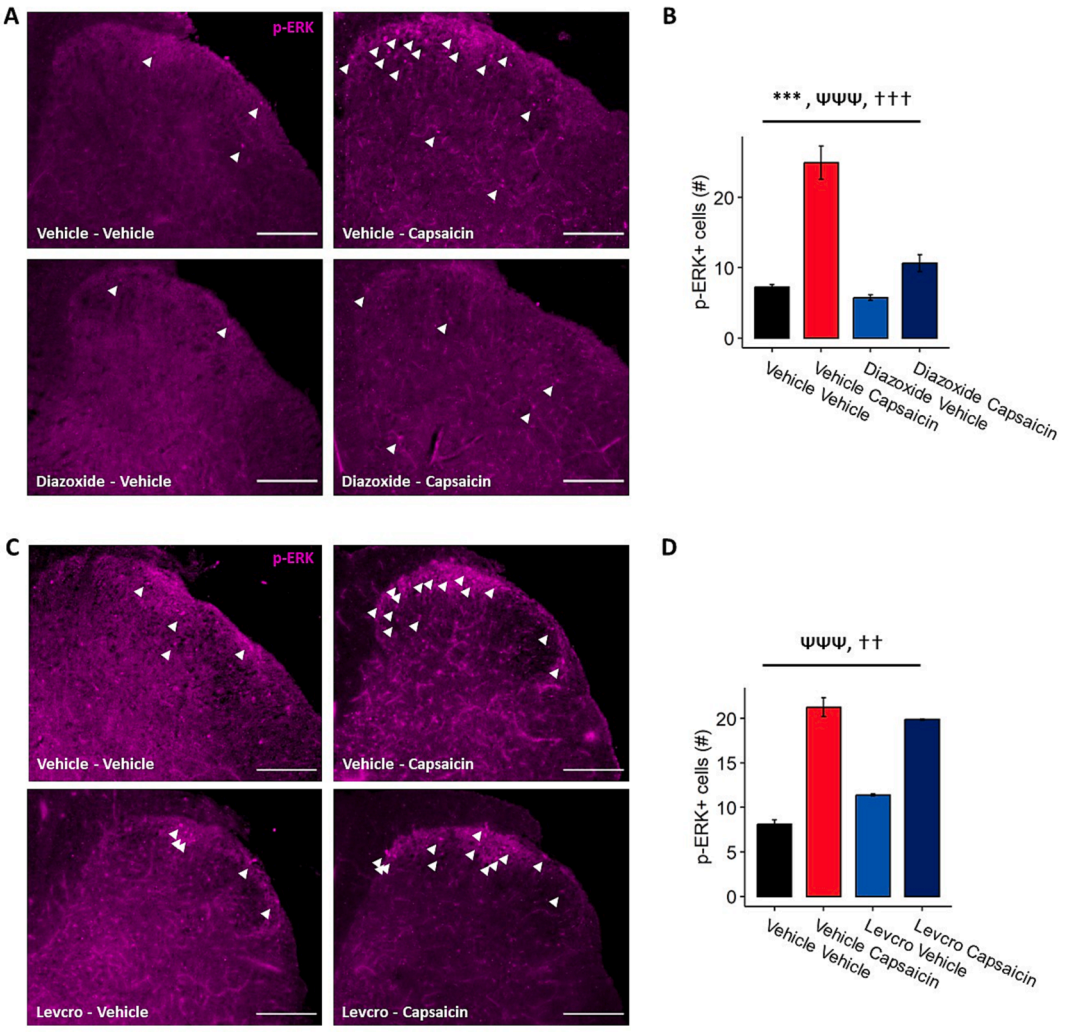


Fig. 8. Diazoxide Reduces p-ERK$^+$ Cell Number in the Spinal Dorsal Horn of Mice Receiving Intraplantar Capsaicin. (*A-D*) Intraplantar capsaicin increased the number of p-ERK$^+$ cells (*A, C, white arrowheads*) in the spinal dorsal horn of mice. (*A-B*) Prior injection of diazoxide prevented spinal neuronal activation, whereas prior injection of levcromakalim (levcro, *C-D*) did not affect increased p-ERK$^+$ cell number. (*B, C*) N-way ANOVA (n = 3/group), *** denotes the effect of noxious stimulus: $p < 0.005$, ΨΨΨ denotes the effect of K_{ATP} channel openers: $p < 0.005$, + denotes the effect of stimulus-K_{ATP} openers interaction: $p < 0.05$, +++ denotes the effect of stimulus-K_{ATP} openers interaction: $p < 0.005$.

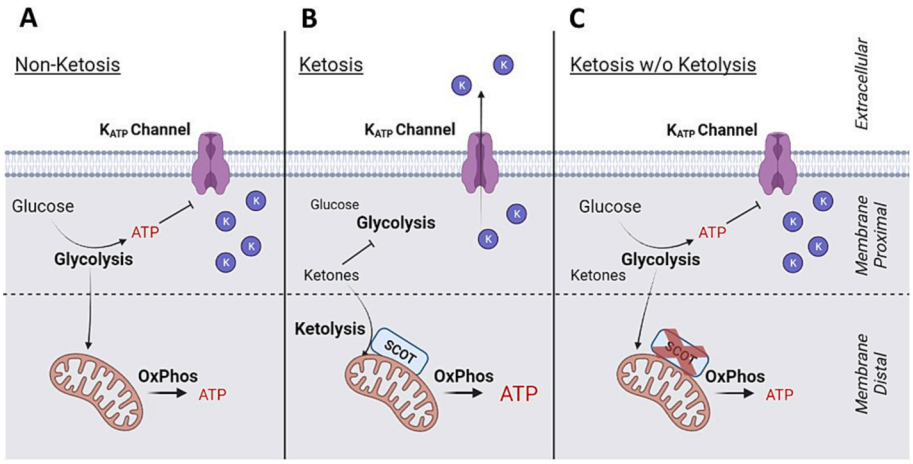


Fig. 9. Ketone Oxidation and K_{ATP} Channel Activity are Required for the Full Analgesic Effect Provided by a Ketogenic Diet. Under normoglycemic, non-ketotic conditions (*A*), glucose is metabolized as fuel by glycolysis. As glycolytic machinery is membrane-bound and associated with K_{ATP} channels (Campanella et al., 2005, Dhar-Chowdhury et al., 2005, Epstein et al., 2014), glycolysis increases membrane-proximal concentrations of ATP and closes K_{ATP} channels. During ketosis and ketolysis (*B*), ketone bodies inhibit glycolysis (Lund et al., 2015) and are oxidized in the mitochondria, decreasing membrane-proximal concentrations of ATP and increasing ATP concentrations further from the membrane. This in turn allows K_{ATP} channel-mediated potassium efflux and hyperpolarization of the cell. During ketosis but in the absence of ketolysis (*C*, SCOT-deficiency), residual dietary carbohydrates are used for glycolysis, again closing K_{ATP} channels and predisposing the cell toward depolarization.

nociception (Fig. 3). These mice lack expression of *Oxct1* in peripheral sensory neurons, which therefore cannot oxidize ketone bodies as fuel. Thus, we reason that the ketogenic diet-related partial protection in these mice is due to methylglyoxal detoxification (Enders et al., 2022b) and ketone oxidation. In a previous study, we reported increased circulating ketones in Adv-KO-SCOT mice fed a ketogenic diet (Enders et al., 2023), indicating that ketone bodies are present to contribute to methylglyoxal scavenging (Enders et al., 2022b) and alternative mechanisms of anti-nociception, including resolution of inflammation (Youm et al., 2015, Shang et al., 2018) and induction of antioxidant response (Wang et al., 2017, Chen et al., 2018, Lu et al., 2018). More importantly, in the absence of ketolysis, sensory neurons in ketogenic diet-fed mice likely utilize the minimal dietary carbohydrates for glycolysis (Fig. 9**C**), as neurons metabolize free fatty acids poorly (Edmond et al., 1987). Glycolysis increases membrane-proximal ATP concentrations, inhibiting K_{ATP} channels and preventing the hyperpolarization of nociceptors, a point that requires further testing in diabetes models.

Our data also suggest that K_{ATP} channels are necessary for a ketogenic diet to provide analgesia. Adding tolbutamide could prevent the antinociceptive activity of a ketogenic diet in response to capsaicin injection. Our data agree with prior studies where treatment with tolbutamide is not pro-nociceptive and, by itself, does not cause allodynia or evoke nocifensive behaviors (Luu et al., 2019). Tolbutamide is a selective inhibitor for K_{ATP} channels containing SUR1 subunits (Ashfield et al., 1999, Babenko et al., 1999), suggesting that SUR1-containing K_{ATP} channels are involved in ketogenic diet-mediated analgesia. Consistent with this view, activating K_{ATP} channels in chow-fed mice with diazoxide mimicked the effects of a ketogenic diet via reductions in pain behaviors and spinal expression of p-ERK. Diazoxide is also a selective agonist of SUR1-containing K_{ATP} channels, having no effect on SUR2A and minimally activating SUR2B-containing K_{ATP} channels (Inagaki et al., 1996, Matsuoka et al., 2000, Wheeler et al., 2008). Importantly, levcromakalim, a K_{ATP} channel agonist that is selective for channels containing SUR2A and SUR2B (Inagaki et al., 1996, Schwanstecher et al., 1998, Matsuoka et al., 2000, Wheeler et al., 2008), did not prevent capsaicin-evoked nociception in the mouse hind paw. We propose a ketogenic diet and ketone oxidation regulate SUR1-containing K_{ATP} channels that alter behavioral responses to a broad spectrum of noxious insults.

The involvement of K_{ATP} channels in antinociception mediated by a ketogenic diet may appear to be at odds with recent studies describing migraine induction following treatment with K_{ATP} channel activators (Al-Karagholi et al., 2019, Christensen et al., 2021) and the ability of a ketogenic diet to reduce migraine (Di Lorenzo et al., 2019, Bongiovanni et al., 2021). In these studies, migraines were induced in patients given subcutaneous levcromakalim, which selectively activates SUR2A and SUR2B-containing K_{ATP} channels (Inagaki et al., 1996, Schwanstecher et al., 1998, Matsuoka et al., 2000, Wheeler et al., 2008). This discrepancy may be related to the selective pharmacology between SUR1- and SUR2-isoform-containing K_{ATP} channel complexes. This view is supported by two pieces of data presented here: 1) tolbutamide, a selective antagonist of SUR1-containing K_{ATP} channels (Ashfield et al., 1999, Babenko et al., 1999), reduced the protective effect of a ketogenic diet against capsaicin, and 2) levcromakalim was unable to prevent capsaicin-evoked nociception or early activation in the spinal dorsal horn.

Additional supportive evidence comes from studies revealing interactions with K_{ATP} channel activity and opioid signaling (Lohmann and Welch, 1999a, Lohmann and Welch, 1999b, Rodrigues and Duarte 2000, Fisher et al., 2019, Sakamaki et al., 2021). SUR1 knockout or SUR1 inhibition reduces opioid-mediated analgesia (Rodrigues and Duarte 2000, Fisher et al., 2019, Sakamaki et al., 2021), and diazoxide-mediated analgesia is partially reduced by knockdown or pharmacological inhibition of μ-, κ-, and δ-opioid receptors (Lohmann and Welch, 1999a, Lohmann and Welch, 1999b). Together with the data presented here, these findings suggest that a ketogenic diet may enhance opioid-mediated analgesia downstream of K_{ATP} channels. As intermittent fasting is associated with increased ketogenesis and ketone oxidation (Cahill, 2006, Cotter et al., 2013, Puchalska et al., 2021), this notion is consistent with reports that intermittent fasting enhances anti-nociception following morphine administration in mice (Duron et al., 2020). Thus, it will be important to explore endogenous opioid production and opioid receptor signaling downstream of ketone oxidation and modulation of K_{ATP} channels in future studies.

Growing evidence suggests that ketogenic diets can play a role in preventing and reversing nociception in preclinical and clinical settings (Ruskin et al., 2009, Cooper et al., 2018b, Di Lorenzo et al., 2019, Bongiovanni et al., 2021, Ruskin et al., 2021, Zhong et al., 2021, Enders et al., 2022a, Enders et al., 2022b, Field et al., 2022). Unfortunately, these diets are restrictive to many, and adherence limits their use. Thus, pharmacologically mimicking the antinociception provided by a ketogenic diet is an attractive therapeutic strategy. Here, we demonstrate that a ketogenic diet provides antinociception to a range of acute noxious stimuli downstream of ketolysis in peripheral sensory neurons. Further, we demonstrate that a ketogenic diet requires K_{ATP} channel activity to mediate this protective effect and that activating K_{ATP} channels without ketosis is sufficient to mimic the antinociception provided by a ketogenic diet. This work suggests targeting SUR1-containing K_{ATP} channels could recapitulate the protective effects of a ketogenic diet without stringent dietary intervention.

Author contributions

JE and DEW designed the research study; JE, JMR, PL, JJ, and ST performed the experiments; JE and DEW analyzed the data; all authors contributed to the manuscript.

CRediT authorship contribution statement

Jonathan D. Enders: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Funding acquisition. **Sarah Thomas:** Investigation, Funding acquisition. **Paige Lynch:** Investigation. **Jarrid Jack:** Investigation. **Janelle M. Ryals:** Investigation. **Patrycja Puchalska:** Methodology. **Peter Crawford:** Methodology, Funding acquisition. **Douglas E. Wright:** Conceptualization, Formal analysis, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: D. E.W. is conducting unrelated research under contract with Annexon Biosciences. P.A.C. has consulted for Pfizer, Inc., Abbott Laboratories, and Jansen Research & Development. The other authors declare no competing financial interests.

Data availability

Data will be made available on request.

Acknowledgments

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