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Loss of SUR1 subtype K_{ATP} channels alters antinociception and locomotor activity after opioid administration

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ABSTRACT

Opioid signaling can occur through several downstream mediators and influence analgesia as well as reward mechanisms in the nervous system. K_{ATP} channels are downstream targets of the μ opioid receptor and contribute to morphine-induced antinociception. The aim of the present work was to assess the role of SUR1-subtype K_{ATP} channels in antinociception and hyperlocomotion of synthetic and semi-synthetic opioids. Adult male and female mice wild-type (WT) and SUR1 deficient (KO) mice were assessed for mechanical and thermal antinociception after administration of either buprenorphine, fentanyl, or DAMGO. Potassium flux was assessed in the dorsal root ganglia and superficial dorsal horn cells in WT and KO mice. Hyperlocomotion was also assessed in WT and KO animals after buprenorphine, fentanyl, or DAMGO administration. SUR1 KO mice had attenuated mechanical antinociception after systemic administration of buprenorphine, fentanyl, and DAMGO. Potassium flux was also attenuated in the dorsal root ganglia and spinal cord dorsal horn cells after acute administration of buprenorphine and fentanyl. Hyperlocomotion after administration of morphine and buprenorphine was potentiated in SUR1 KO mice, but was not seen after administration of fentanyl or DAMGO. These results suggest SUR1-subtype K_{ATP} channels mediate the antinociceptive response of several classes of opioids (alkaloid and synthetic/semi-synthetic), but may not contribute to the "drug-seeking" behaviors of all classes of opioids.

1. Introduction

Classical opioid analgesia works through G-protein coupled receptor (GPCR) signaling, followed by a decrease in Ca^{2+} activity and an increase in K^+ signaling through direct or indirect $G_{\alpha i}$-protein modulation [1]. Activation of potassium channels such as inwardly-rectifying potassium channels (K_{ir}) and G-protein coupled inwardly-rectifying potassium channels (GIRKs) are suggested as potential targets for broadening analgesic therapies through ligand bias at opioid receptors [2,3]. Previous studies indicate buprenorphine and DAMGO signal through K_{ir} [4], but other synthetic opioids such as fentanyl may not share the same signaling pathways [5]. Research into various signal transduction pathways and targets of opioids are ongoing in search of novel targets for pain relief. For example, TRV130 and PZM21, were developed specifically to decrease the adverse effects of opioids by targeting the μ-opioid receptor and G-protein activation without recruiting β-arrestin signal transduction pathways [6–9].

Among the K_{ir}s, ATP sensitive potassium channels (K_{ATP}) are a downstream target of the μ opioid receptor [10] and produce analgesia in rodent pain models [11–14]. Previous studies indicate activation of K_{ATP} channels potentiates morphine analgesia [15], while loss of K_{ATP} channel subunit expression decreases morphine antinociception [16]. K_{ATP} channels are found in the peripheral nervous system, spinal cord, and brain [17,18] and form an octamer which consists of four Kir6.X subunits and four regulatory sulfonylurea (SUR) subunits [17,19]. Kir6.X and SURX are also expressed in pairs, with different combinations being more prominent in certain tissues, such as Kir6.2/SUR1 in the peripheral nervous system [17,20], Kir6.1/SUR1 and Kir6.1/SUR2 in the spinal cord [21], and various combinations of K_{ATP} channel subunits across the brain depending on region.

The administration of drugs of abuse, including opioids, increase locomotor activity in rodents which is thought to be correlated to drug

Abbreviations: AAV, associated adenovirus; ANOVA, analysis of variance; DAMGO, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin; DRG, dorsal root ganglia; FLIPR, fluorescence intensity plate reader; G-protein coupled inwardly-rectifying potassium channel, GIRK channel; HBSS, Hank's Buffered Salt Solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; K_{ATP} Channel, ATP-sensitive potassium channel; Kir, inwardly-rectifying potassium channels; KO, knock out; PCR, polymerase chain reaction; SUR1, sulfonylurea receptor 1; WT, wild type.

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seeking behaviors. Behavioral studies indicate that modulation of K_{ATP} channel activity affects these behaviors in rodents. Iptakalim, a K_{ATP} channel opener, was found to attenuate nicotine-induced dopamine and glutamate release in the nucleus accumbens of rats [22,23], and systemic administration of the K_{ATP} channel agonists iptakalim, cromakalim, and pinacidil decreases amphetamine induced hyperlocomotion [24,25]. These data suggest activation of K_{ATP} channels in the central nervous system can also attenuate drug seeking behaviors in addition to nociception. Whether an attenuation of antinociception and a potentiation of hyperlocomotion are found after administration of synthetic and semi-synthetic opioids after loss of K_{ATP} channel activity is not known at this time.

In this study, opioid-induced behaviors were measured using mechanical and thermal sensitivity and open field testing, to investigate antinociceptive and locomotor effects of buprenorphine, DAMGO, and fentanyl in SUR1-deficient mice, respectively. Attenuation of antinociception after buprenorphine, DAMGO, and fentanyl administration was found in SUR1 knock out (KO) compared to wildtype (WT) mice, but hyperlocomotion was only largely potentiated after morphine or buprenorphine administration. These data suggest several opioids can stimulate K_{ATP} channel activation to induce analgesia, but K_{ATP} channel activity with regards to drug seeking or other opioid-induced behaviors may be dependent on the opioid ligand used, possibly due to differences in downstream signaling cascades across the nervous system.

2. Materials and methods

2.1. Experimental models and design

All experimental procedures involving animals were approved and performed in accordance with the University of Minnesota Institutional Animal Care and Use Committee. Breeding pairs of SUR1-deficient mice (SUR1 KO) and $SUR1^{flx/flx}$ (SUR1 flox) mice were obtained from the laboratory of Dr. Joseph Bryan at the Pacific Northwest Research Institute (Seattle, WA, United States) as used in previous studies [26,27] and kept on a C57Bl6N background. SUR1 WT littermates were used as controls to SUR1 KO mice. Behavioral experiments were performed on adult male and female mice (>5 weeks of age, 20−40 g) and tissue isolation was performed on adult mice (4–8 weeks, 20−40 g). Genotype was verified by PCR as in previous studies [11,26].

In one set of experiments, SUR1 KO and SUR1 WT mice were tested for acute antinociception to opioid analgesics in both mechanical and thermal paw withdrawal threshold assays. In a separate set of experiments, SUR1 KO and SUR1 WT mice were tested for changes in locomotor activity after opioid administration. SUR1 flox mice were also tested separately for changes in locomotion after morphine administration. Dorsal root ganglia (DRG) and spinal cord dorsal horn cells were harvested from separate SUR1 KO and SUR1 WT animals for potassium flux assays. Experiments were blinded to the experimenter; however blinding was not possible for SUR1 KO mice as these mice have white fur and the WT littermates have black fur.

2.2. Drugs

Buprenorphine (5.83 mg/kg; PHR172, Sigma Aldrich, St. Louis, MO), DAMGO (10 mg/kg; Bachem, Bubendorf, Switzerland), morphine (5 and 15 mg/kg; Spectrum Chemical, New Brunswick, NJ), and fentanyl (0.25 mg/kg; F2886, Sigma Aldrich) were diluted in saline (vehicle) and administered through 100 μL subcutaneous injections. The drug concentrations used on this study were chosen to achieve similar antinociceptive effects in naïve mice, and are similar to those found in previous studies in rodents for buprenorphine [28,29], fentanyl [30], and DAMGO [31]. Some mice were used multiple times. To avoid lingering effects from previous drug injections and tests, a minimum of three days elapsed between each injection and test. Drug and test order were randomized using online random number generation programs.

2.3. Mechanical paw withdrawal

Mice were acclimated on several separate occasions in individual acrylic containers on a shared wire mesh floor one week prior to the start of experimentation. Mechanical paw withdrawal thresholds were measured using electronic von Frey testing equipment (Electric von Frey Anesthesiometer, 2390, Almemo® 2450, IITC Life Science, Woodland Hills, CA) [32]. Thresholds were measured as the amount of force in grams required to elicit a nocifensive response on the plantar surface of both rear hind paws. Baseline thresholds consisted of the average of 3–5 measurements from each hind paw prior to drug administration. Post-drug administration thresholds were measured once from each hind paw at 3, 15, 30, 45, and 60 min post-injection. Post-drug thresholds were also measured at 120 min after drug administration for buprenorphine, which has a slower onset and longer duration of analgesia than fentanyl or DAMGO.

2.4. Thermal paw withdrawal

Mice were acclimated on multiple separate occasions to individual acrylic containers on a shared glass floor heated to 30℃ one week prior to the start of each experiment. Thermal paw withdrawal latencies were measured using a modified Hargreaves Method (Plantar Test Analgesia Meter, 400, IITC, Woodland Hills, CA) [33]. Latencies were measured as the amount of time in seconds for a heat radiant light beam focused on the plantar surface of each hind paw required to elicit a nocifensive response [34]. A time limit of 20 s of exposure to the beam was implemented to avoid tissue damage. Mice were restrained using laboratory-made restrainers. Baseline latencies were calculated as the average of 3 measurements per hind paw prior to drug administration. Post-drug latencies were taken post-drug administration at the same time points as mechanical testing.

2.5. Primary culture of DRG and spinal cord dorsal horn cells

Animals were anaesthetized with 5% isoflurane in oxygen and decapitated prior to tissue isolation. DRG isolation was similar to previous reports but are summarized below [16]. DRG were extracted from male SUR1 WT and SUR1 KO mice (4–8 weeks, 20–40 *g*) and placed on ice in Petri dishes containing 1X Hank's Balanced Salt Solution (HBSS, SH30588.02, GE Healthcare Life Sciences, US). Ganglia were minced using surgical micro scissors and incubated in a papain solution (32 μL papain (27.3 U/mg, no. 3126; Worthington Biochemical, Lakewood, NJ, US) with 1 mg l-cysteine, (Sigma Aldrich) in 1.5 mL 1X HBSS in a 37 °C rocking water bath for 10 min. The samples were then centrifuged at 1600 rpm for 2 min (Eppendorf 5415R, 200xg) and the supernatant aspirated before the tissue was incubated in a collagenase solution containing 2 mg/mL collagenase type II (CLS2; Worthington Biochemical) in 5 mL 1X HBSS in a 37 °C rocking water bath for 10 min. Cells were centrifuged at 1500 rpm for 2 min (Beckman Allegra X15R, 524 x g), supernatant was removed and tissue was carefully triturated using fire-polished glass pipettes with 2 mL pre-warmed media. The media contained Eagle's Minimum Essential Medium with Earle's salts and L-glutamine (10−010-CV; Corning®, Corning, NY, US), 10 % v/v horse serum (26050−088; Gibco, Thermo Fisher Scientific, Waltham, MA, United States), 1% v/v 100X MEM vitamins solution (11120−052; Gibco, Thermo Fisher Scientific, Waltham, MA, United States) and 1% v/v penicillin-streptomycin (15140−122; Gibco, Thermo Fisher Scientific, Waltham, MA, United States). DRG were then centrifuged for 2 min at 1000 rpm (Beckman Allegra X15R, 233 x g), supernatant aspirated, and the pellet suspended in 5 mL pre-warmed media. Isolated DRG were plated in 100 μL aliquots into clear bottom, black-walled 96-well plates coated with 2.5 μg/cm^2 poly-D-lysine (354,210; Corning®, Corning, NY, US) and incubated in a humidified incubator at 37 °C with 5% CO_2 (~1.5 × 10^5 cells/well).

Spinal cord dorsal horn cell isolation was modified from a previous

report [35]. To isolate spinal cord dorsal horn cells, the spinal cords from SUR1 WT and SUR1 KO mice (4–8 weeks, 20–40 g) were placed on ice in Petri dishes containing 10 mM HEPES (BP310−1; Thermo Fisher Scientific, Waltham, MA, United States) in 1X Hank's Balanced Salt Solution (SH30588.02; GE Healthcare Life Sciences, United States). The dura was removed from the spinal cord and was bisected lengthwise. The halves were bisected again and a quarter width of the spinal cord was cut off the lateral most edge and placed in 1X HBSS. The dorsal horn strips were digested in a papain solution (45 μL papain, no. 3126; Worthington Biochemical, Lakewood, NJ, United States in 3 mL 1X HBSS) for 30 min at 37 °C with 5% CO2, swirling every 5 min. The supernatant was removed and tissue was washed twice with 1 mL ice cold HEPES/HBSS solution. The tissue was then washed with 1 mL pre-warmed media consisting of Eagle's Minimum Essential Medium with Earle's salts and L-glutamine (10−010-CV, Corning®, Corning, NY, United States), 10 % v/v horse serum (26050−088; Gibco, Thermo Fisher Scientific, Waltham, MA, United States), 1% v/v 100X MEM vitamin solution (11120−052; Gibco, Thermo Fisher Scientific, Waltham, MA, United States), and 1% v/v penicillin-streptomycin (15140−122; Gibco, Thermo Fisher Scientific, Waltham, MA, United States). The supernatant was then removed and the tissue was triturated with 2 mL pre-warmed media using a fire-polished Pasteur pipette and centrifuged at 1000 x g for 5 min. The supernatant was removed and the pellet was suspended in 3 mL pre-warmed media and plated at ~1.2×10^5 cells/well.

2.6. Fluorescence intensity plate readings

Fluorescence intensity plate reading (FLIPR) assays were performed on cultured DRG and spinal cord dorsal horn sensory neuron cells 24 h post isolation. Potassium flux of cells was measured using FLIPR Potassium Assay Kit (Molecular Devices, LLC, San Jose, CA) according to manufacturer instructions [36]. Cell culture media was replaced with a 1:1 mixture of 1X HBSS (SH30588.02, GE Healthcare Life Sciences, United States) with 20 mM HEPES (BP310−1, Thermo Fisher Scientific, Waltham, MA, United States) and FLIPR Loading Dye with 5 mM probenecid. Cells were incubated for 1 h at room temperature in the dark before opioids were added (10 μL, in saline vehicle) and incubated for an additional 10 min. A nine-point dose response curve (10^{-4} M to 10^{-12} in saline) was collected in addition to saline controls. Fluorescence data was collected after the addition of 60 μL of 10 mM thallium sulfate solution per well. Background fluorescence was measured for 30 s in 21 s intervals before addition of opioids and after 10 min opioid incubation. Fluorescence post-thallium addition was monitored every 21 s for 600 s total using a BioTek Synergy 2 (BioTek, Winooski, VT, United States) multi-well plate reader equipped with an excitation filter of 485/20 nm and emission filter 528/20 nm. Four technical replicates were performed for each drug concentration and internal controls.

2.7. Open field testing

Mouse activity was measured after placement in the center of a 40 cm × 40 cm open field arena with a camcorder situated above enclosure (HDR-CX405; Sony Corp., Tokyo, Japan). Equal light distribution (45−65 lux) in the arena was verified using a digital light reader. After a 15 min baseline recording without any drug, opioids were administered and behavior was recorded for an additional 30 min. Recordings were analyzed offline using Ethowatcher® (developed by the Laboratory of Comparative Neurophysiology of the Federal University of Santa Catarina, freely available on www.ethowatcher.ufsc.br, IEB-UFSC [37]) which translates movement of the animal for frame-by-frame analysis. The parameters used for comparison across groups were distance traveled, time spent immobile, velocity, and total change in angular direction (in degrees).

2.8. Adeno-Associated virus serotype 9 (AAV9)-mediated SUR1 knockdown

AAV9-mediated Cre expression in SUR1 flox mice was achieved by intrathecal injection of either AAV9-hSyn-GFP-Cre or AAV9-hSyn-GFP-ΔCre (10 μL containing ~10^{13} vector genomes, University of Minnesota Viral Vector Core, Minneapolis, MN, United States) [38]. Verification of mRNA knockdown was achieved using quantitative polymerase chain reaction and histological sections were taken from some animals in order to demonstrate successful delivery of AAV vectors by visualization of green fluorescent protein [16].

2.9. Data analysis

Mechanical and thermal paw withdrawal data was analyzed for differences between genotypes using one-factor repeated measures ANOVA and two-factor repeated measures ANOVA for genotype and sex differences. *In vitro* fluorescence data was obtained by summing the fluorescence intensities for each opioid concentration over the 2 min period following the addition of thallium compared to a control well without drug. Total fluorescence was compared between SUR1 KO and WT mice using the Mann-Whitney *U* test The open field testing measurements obtained post opioid administration were separated into five-minute bins with differences between genotype and sex analyzed using repeated measures ANOVA. No significant differences were seen between male and female mice during behavioral tests, so these data were pooled. All data were analyzed using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA), p-values < 0.05 were considered significant. Data are presented as wither mean ± SEM or as medians with 95 % confidence intervals as appropriate. Sample size calculations were based off our previous study, which suggests a corrected effect size of ~2 (Hedge's g_s), assuming for a two-tailed hypothesis, $\alpha = 0.05$, and power ≥ 80 % [39].

3. Results

3.1. Mice lacking SUR1 subtype K_{ATP} channels have altered antinociception after buprenorphine, fentanyl, or DAMGO administration

Mice deficient in SUR1-type K_{ATP} channels display decreased morphine mechanical antinociception [16]. To compare the antinociceptive effects of synthetic and semi-synthetic opioids including buprenorphine, fentanyl, and DAMGO, mice lacking the SUR1 regulatory subunit of K_{ATP} channels (SUR1 KO) were subjected to mechanical paw withdrawal threshold testing. Administration of either buprenorphine (Fig. 1A), fentanyl (Fig. 1B) or DAMGO (Fig. 1C) increased mechanical thresholds post-drug administration. Mechanical thresholds were significantly attenuated in SUR1 KO mice after buprenorphine (unpaired t-test, $p = 0.0002$), fentanyl (unpaired t-test, $p = 0.0018$), and DAMGO (unpaired t-test, $p = 0.0109$) treatment over the entire time course post-injection compared to WT mice (Fig. 1D). In a previous study, mechanical nociception was attenuated with morphine administration, but thermal nociception was not as significantly affected in SUR1-type K_{ATP} deficient mice [16]. We observed attenuation in thermal withdrawal latencies after buprenorphine administration (Fig. 1E), but not after fentanyl or DAMGO injection (Fig. 1F-G). Similar to the mechanical threshold data, thermal withdrawal latencies were significantly attenuated in SUR1 KO mice after buprenorphine (Fig. 1H, unpaired t-test, $p = 0.0210$). Saline injection did not significantly alter mechanical nor thermal antinociception (Supplemental Fig. 1). The loss of K_{ATP} channel activity in the nervous system attenuates mechanical antinociception in synthetic and semi-synthetic opioids, similar to morphine.

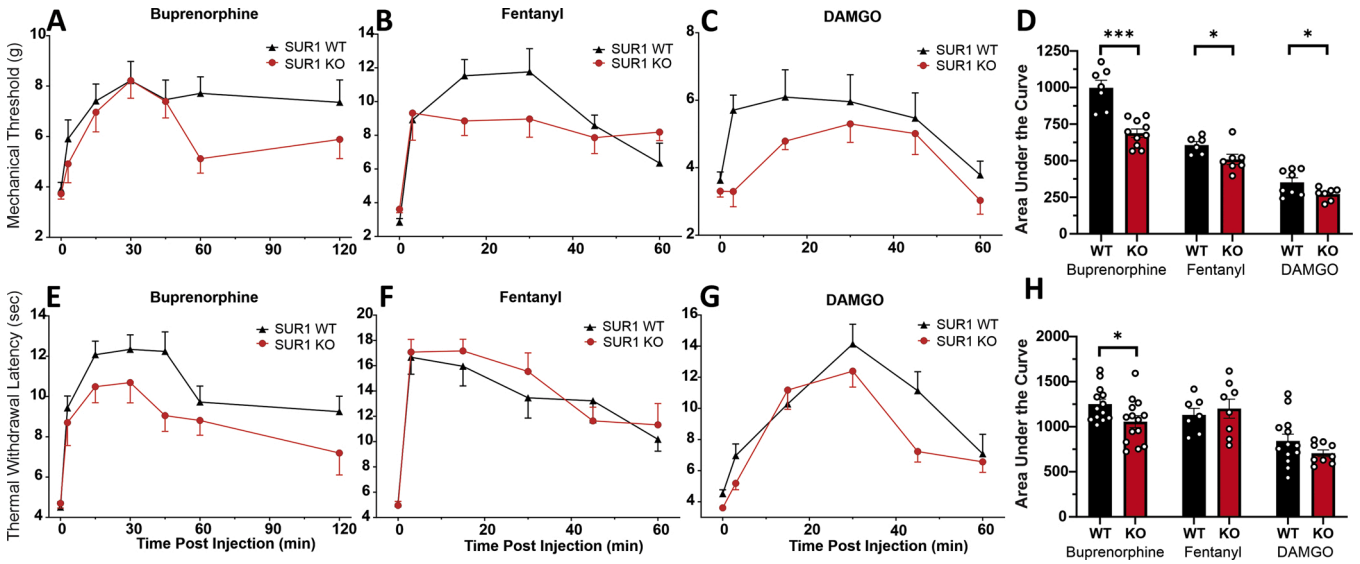

Fig. 1. Mice lacking SUR1 subtype K_{ATP} channels have decreased mechanical antinociception after synthetic and semi-synthetic opioid administration. Baseline mechanical paw withdrawal thresholds were measured for all SUR1 WT and SUR1 KO mice prior to and after administration of (A) buprenorphine (5.83 mg/kg, s.c.), (B) fentanyl (0.25 mg/kg, s.c) and (C) DAMGO (10 mg/kg, s.c.) over 60 or 120 min. A significant interaction was found between genotype x time after fentanyl administration (B, repeated measures ANOVA, $F(5, 55) = 2.415$, $p = 0.0474$). Area under the curve (D) for buprenorphine over 120 min (unpaired t-test, $p = 0.0002$), fentanyl (unpaired t-test, $p = 0.0018$), and DAMGO over 60 min (unpaired t-test, $p = 0.0109$) for mechanical threshold data indicate a significant decrease in thresholds of SUR1 KO mice compared to SUR1 WT mice over the entire time course post-injection. Thermal paw withdrawal latencies were measured for SUR1 WT and SUR1 KO mice prior to and after administration of (E) buprenorphine (5.83 mg/kg, s.c.), (F) fentanyl (0.25 mg/kg, s.c.) and (G) DAMGO (10 mg/kg, s.c.) over 60 or 120 min. A significant genotype effect was found after buprenorphine administration (E, repeated measures ANOVA, $F(1, 25) = 6.07$, $p = 0.021$). Area under the curve (H) for buprenorphine (unpaired t-test, $p = 0.0210$) indicates a significant decrease in latencies of SUR1 KO mice compared to SUR1 WT mice over the entire course of testing post-injection. * and *** represent significant differences ($P < 0.05$ and $P < 0.001$ respectively). Each curve represents the mean± SEM response of 7-13 male and female mice.

3.2. Potassium flux is decreased in DRG and spinal cord dorsal horn cells from SUR1 KO mice after acute buprenorphine or fentanyl exposure

A global loss of SUR1-subtype K_{ATP} channels potentiates mechanical hypersensitivity in mice and attenuates morphine antinociception [11]. K_{ATP} channels are expressed in DRG and in spinal cord dorsal horn [20], and are therefore a possible site of analgesic action of opioids. Localized deletion of the same K_{ATP} channel subtype in the lumbar dorsal horn and DRG also potentiate mechanical hypersensitivity, and decrease potassium flux in DRG after chronic morphine exposure [16]. Potassium flux was similarly examined after acute exposure to buprenorphine, fentanyl, and DAMGO in DRG and spinal cord dorsal horn isolations. DRG and spinal dorsal horn cells were isolated from SUR1 KO and SUR1 WT mice and these cells were exposed to ascending concentrations of either buprenorphine, fentanyl or DAMGO and fluorescence was measured over the course of 2 min (Fig. 2). Overall, potassium flux was lower in SUR1 KO DRG following acute exposure to buprenorphine, fentanyl, and morphine compared to DRG from SUR1 WT mice (Fig. 2A, Mann Whitney U, $p = 0.0286$). Potassium flux was also decreased in SUR1 KO spinal dorsal horn cells after acute buprenorphine and fentanyl administration (Fig. 2B, Mann Whitney U, $p = 0.0286$).

3.3. Hyperlocomotion after acute administration of morphine and buprenorphine is potentiated in mice lacking SUR1 subtype KATP channels

Systemic administration of morphine or other drugs of abuse can lead to "drug-seeking" behaviors, including hyperlocomotion. In order to examine acute opioid-induced hyperlocomotion, open field tests examined distance traveled, time spent immobile, velocity, and the total change in orientation angle [40]. Locomotor activity has been shown to

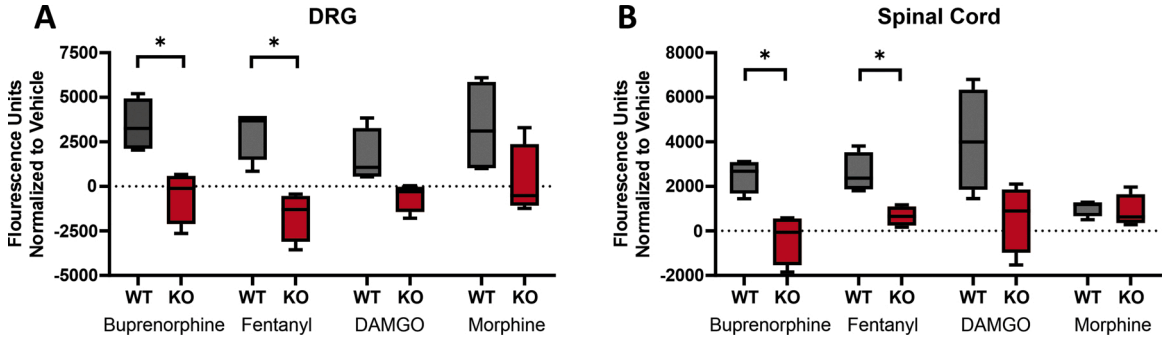

Fig. 2. Potassium flux is decreased in DRG and spinal cord dorsal horn cells from SUR1 KO mice after acute opioid treatment. Fluorescence was pooled across a nine-point dose response curve and averaged across four trials from either SUR1 WT or KO mice. (A) Potassium flux in DRG is significantly attenuated after acute administration of buprenorphine (Mann–Whitney *U*, $p = 0.0286$) and fentanyl (Mann–Whitney *U*, $p = 0.0286$). (B) Potassium flux in spinal cord dorsal horn cells is significantly attenuated after acute administration of buprenorphine (Mann–Whitney *U*, $p = 0.0286$) and fentanyl (Mann–Whitney *U*, $p = 0.0286$). Minimum to maximum values and median values shown in the box-and-whiskers plots.

increase after morphine administration and decrease after morphine is withdrawn, and is sometimes used as a pseudo-indicator of drug-seeking activity in rodents [41,42]. The hypothesis that a loss of function of K_{ATP} channels would change novelty seeking behaviors was tested. After administration of 5 mg/kg morphine, the distance traveled within an open field arena was significantly higher for SUR1 KO compared to SUR1 WT mice (Fig. 3A; repeated measures ANOVA, F (1, 12) = 4.957, p = 0.0459). The time spent immobile was also significantly decreased for SUR1 KO (mice compared to SUR1 WT mice (Fig. 3B; repeated measures ANOVA, F (1, 12) = 5.240, p = 0.0410). Animal velocity was not significantly different between the treatment groups (Fig. 3C), but the change in angular orientation was significantly increased in SUR1 KO compared to SUR1 WT mice (Fig. 3D, repeated measures ANOVA, F (1, 12) = 10.16, p = 0.0078). A dose of 15 mg/kg morphine resulted in similar findings including a significant increase in distance traveled (Fig. 3E, repeated measures ANOVA, F (1, 28) = 7.752, p = 0.0095) and a significant decrease in time spent immobile (Fig. 3F, repeated measures ANOVA, F (1, 28) = 13.11, p = 0.0012) in SUR1 KO compared to SUR1 WT mice. The speed and change in angular orientation were not significantly different across genotypes (Fig. 3G-H). Spatially restricted loss of SUR1-subytpe KATP channels by intrathecal injection of AAV9-hSyn-Cre in SUR1 flox mice did not significantly change locomotion compared to control animals, indicating ascending projections do not substantially contribute to hyperlocomotion after morphine administration (Supplemental Fig. 2).

In the current and previous study, SUR1 KO mice have a loss of mechanical and thermal antinociception after acute morphine and buprenorphine administration, and hyperlocomotion was potentiated in SUR1 KO animals after morphine administration. Therefore, the ability of buprenorphine to potentiate hyperlocomotion was tested. The distance traveled after buprenorphine administration was significantly higher in SUR1 KO mice compared to SUR1 WT mice (Fig. 4A; repeated measures ANOVA, interaction: F (6, 192) = 3.331, p = 0.0038). Conversely, time spent immobile was also significantly decreased in SUR1 KO mice compared to SUR1 WT mice (Fig. 4B; repeated measures ANOVA, interaction: F (6, 192) = 2.387, p = 0.0301). Both animal velocity (Fig. 4C) and change in angular orientation (Fig. 4D) of SUR1 KO mice were significantly higher compared to SUR1 WT mice (Fig. 4C; repeated measures ANOVA, F (6, 192) = 5.194, p < 0.0001, Fig. 4D; repeated measures ANOVA, F (6, 192) = 3.130, p = 0.006).

Locomotion after administration of fentanyl was not significantly altered between SUR1 KO and SUR1 WT animals (Figs. 4E–H). After DAMGO treatment, the distance traveled was significantly higher for SUR1 KO mice compared to SUR1 WT mice over time (Fig. 4I, repeated measures ANOVA, F (6, 198) = 2.899, p = 0.0099). Other measurements including time spent immobile, velocity, and change in angular orientation were not significantly different after DAMGO administration (Fig. 4J-L). Overall, these data indicate loss of SUR1 subtype K_{ATP} channels affect behavioral changes attributed to opioids, in addition to their analgesic qualities.

4. Discussion

In a previous study, loss of the SUR1 subunit of the K_{ATP} channel either by global knockout, intrathecal injection of shRNA targeting the *Abcc8* gene or conditional deletion using Cre-lox recombination, attenuated mechanical antinociception after morphine administration [16]. Data presented here investigated if buprenorphine, fentanyl, and the μ opioid receptor (MOR) specific agonist, DAMGO, would also produce similar effects to morphine. Two stimulus-evoked tests were used in this study to measure opioid-induced antinociception, namely the modified Hargreaves method for thermal sensitivity and von Frey method for mechanical sensitivity. This data is in agreement with previous studies finding the potentiation of morphine analgesia with concurrent use of K_{ATP} channel agonists [15,43]. Data presented here also demonstrate global SUR1-deficiency attenuated the antinociception after administration of buprenorphine, DAMGO, and fentanyl. However, thermal sensitivity did not seem to be affected to the same magnitude, concurrent with previous studies using morphine [16]. SUR1-defiency also prolonged the increase in locomotor activity after morphine and buprenorphine administration, indicating the global loss of SUR1-subtype K_{ATP} channel leads to a lack of inhibition of opioid signaling in the brain, leading to prolonged reward-seeking behavior.

Buprenorphine is a semi-synthetic derivative of an opiate alkaloid. The antinociceptive effects of buprenorphine are due to partial activation of MORs with high affinity and low intrinsic activity [28]. Previously, buprenorphine has shown bias against MOR phosphorylation and β-arrestin recruitment while super-activating adenylyl cyclase at certain doses [44–47]. Buprenorphine has a slow dissociation from the MOR, and can displace other full μ-agonists such as morphine, which is useful to treat drug dependence in the clinic. Since morphine and buprenorphine share similar chemical structures, it is not surprising the

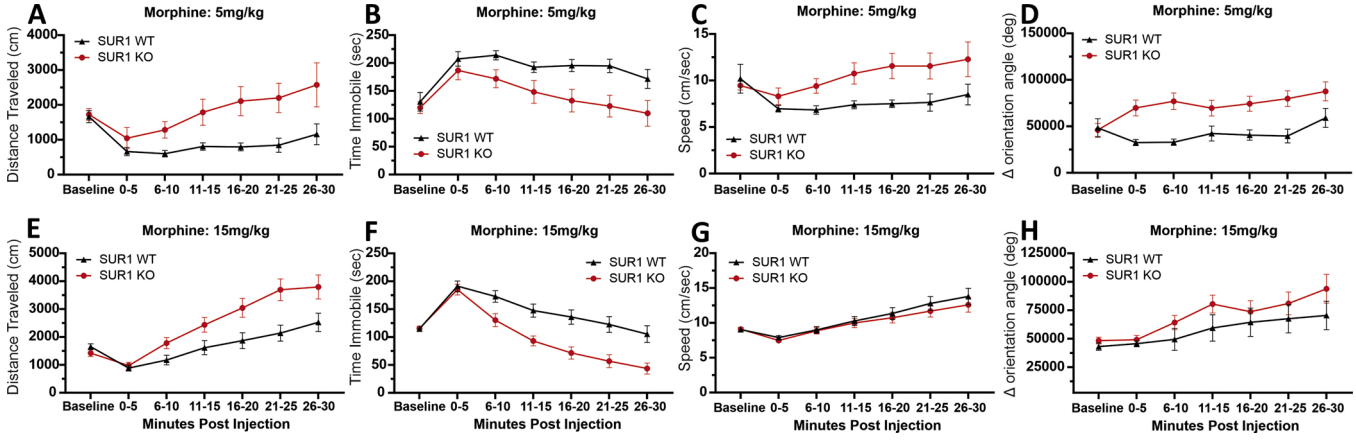

Fig. 3. Hyperlocomotion after acute administration of morphine is potentiated in SUR1 KO mice. After a 15 min baseline analysis, locomotion parameters were measured in mice following injection of either 5 mg/kg (A–D) or 15 mg/kg morphine (E–H). SUR1 KO mice had a significantly higher distance traveled (A) than SUR1 WT after 5 mg/kg (factor: genotype, F (1, 12) = 4.957, p = 0.0459) and (E) 15 mg/kg morphine (factor: genotype, F (1, 28) = 7.752, p = 0.0095). The time spent immobile was significantly lower in SUR1 KO mice compared to SUR1 WT mice after (B) 5 mg/kg (factor: genotype, F (1, 12) = 5.240, p = 0.0410) or (F) 15 mg/kg morphine (factor: genotype, F (1, 28) = 13.11, p = 0.0012). The overall velocity (C, G) was not significantly different between SUR1 KO and SUR1 WT mice, but the change in angular orientation was significantly higher in SUR1 KO compared to SUR1 WT mice after (D) 5 mg/kg morphine (factor: genotype, F (1, 12) = 10.16, p = 0.0078) but not 15 mg/kg (H). Data analyzed by repeated measures ANOVA. Each curve represents the mean ± SEM response of 5-9 male and female mice per group in A–D and 15 male and female mice per group in E–H.

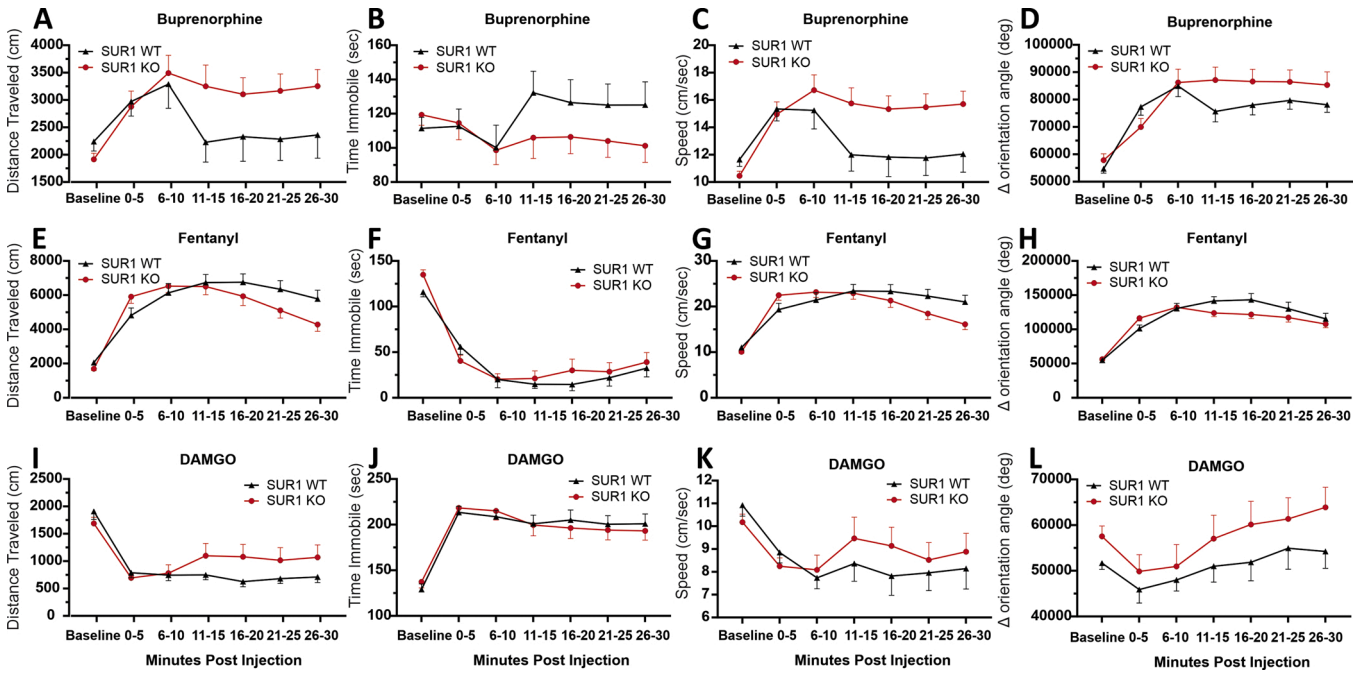

Fig. 4. Hyperlocomotion after acute administration of buprenorphine is potentiated in SUR1 KO mice. After a 15 min baseline analysis, locomotion parameters were measured in mice following injection with either buprenorphine (5.83 mg/kg, s.c., A–D), fentanyl (0.25 mg/kg, s.c., E–H) or DAMGO (10 mg/kg, s.c., I–L). The distance traveled in an open field (A) was significantly higher for SUR1 KO compared to SUR1 WT mice (interaction: genotype x time, $F(6, 192) = 3.331$, $p = 0.0038$) and (B) the amount of time spent immobile was significantly lower in SUR1 KO mice compared to SUR1 WT mice (interaction: genotype x time, $F(6, 192) = 2.387$, $p = 0.0301$) after buprenorphine injection. The overall velocity (C) and the (D) change in angular orientation was significantly higher in SUR1 KO compared to SUR1 WT mice after buprenorphine administration (C; interaction: genotype x time, $F(6, 192) = 5.194$, $p < 0.0001$) (D; interaction: genotype x time, $F(6, 192) = 3.130$, $p = 0.006$). There was no change in locomotor activity after administration of fentanyl (E–H). SUR1 KO mice had a significantly higher (I) distance traveled (interaction: genotype x time, $F(6, 198) = 2.899$, $p = 0.0099$) compared to SUR1 WT mice after DAMGO administration. Data analyzed by repeated measures ANOVA. Each curve represents the mean ± SEM response of 17 male and female mice per group in A–D, 17-18 male and female mice per group in E–L.

antinociceptive and hyperlocomotive actions of both drugs were altered in SUR1 KO mice compared to WT controls. Although buprenorphine has not been shown to induce K_{ATP} channel currents *in vitro*, the antinociceptive effects of intracerebral ventricle (icv) buprenorphine and morphine are lost after systemic K_{ATP} channel inhibition *in vivo* [48]. Therefore it is not surprising that a global loss of SUR1-subtype K_{ATP} channel activity significantly modified both the antinociceptive and locomotor activities of mice after buprenorphine treatment.

The loss of SUR1 subtype K_{ATP} channels did attenuate antinociception and but did not exacerbate hyperlocomotion after fentanyl or DAMGO administration. Fentanyl is used clinically, particularly during perioperative procedures, as a potent synthetic μ-receptor–stimulating opioid. The analgesic and euphoric effects of fentanyl are largely attributed to activation of the MOR, however effects are seen at alternate opioid receptors [49]. Fentanyl's ligand bias at MORs has been extensively characterized, and the overexpression of GPCR kinase and β-arrestin2 after fentanyl administration has been reported to enhance desensitization of μ-opioid-mediated GIRK potassium flux [50,51]. However, it is unknown if fentanyl can indirectly activate or desensitize K_{ATP} channels, as seen with GIRK channels. DAMGO is also a synthetic opioid with high MOR specificity and was invented as a biologically stable analog of enkephalin but has little clinical use due to severe respiratory depression and sedation. There is almost no information on the effects of DAMGO on the activity of K_{ATP} channels in neurons, but DAMGO is reported to inhibit K_{ATP} channels in cardiomyocytes expressing subunits other than SUR1 [52]. These data indicate the behavioral effects seen after systemic administration of different opioids are through different effector mechanisms, which may be dependent on cell type or location.

An open field test was used to measure locomotive behavior after opioid exposure as performed in previous studies [32,33,40,53,54]. Previously, a loss of Kir6.2 in mice was shown to produce hyperlocomotion after NMDA receptor inhibition [55]. Conversely, systemic administration of the K_{ATP} channel agonists, iptakalim, cromakalim, and pinacidil decreases amphetamine-induced hyperlocomotion [24,25]. Although the neural circuitry behind these observations has not been thoroughly investigated, it is possible drugs of abuse facilitate activation of neuronal circuits by altering K_{ATP} channel activity. Alteration of K_{ATP} channel activity has been shown to lead to enhanced dopamine release and activation of circuits involved in addiction [56,57] or indirectly through disinhibition of the ventral tegmental area through the ventromedial hypothalamus [58,59]. Interestingly, the administration of K_{ATP} channel agonists either icv or systemically reduces morphine withdrawal in rodents [60,61], indicating the loss of K_{ATP} channel activity may have additional roles in brain circuitry related to drug dependence. Behavioral data presented here after morphine and buprenorphine administration would support this hypothesis and previous findings with other drugs of abuse.

Behavioral data presented here in SUR1 global knockout mice after the systemic administration of structurally different opioid agonists were strikingly similar, but with some differences. Mechanically evoked thresholds were significantly attenuated in the SUR1-deficient mice for all opioids tested. This is in line with previous studies which found fentanyl analgesia could be attenuated by intraplantar administration of K_{ATP} channel antagonists [62], and icv administration of the K_{ATP} channel agonist cromakalim could enhance antinociception produced by buprenorphine and morphine [48]. Hyperlocomotion was potentiated in SUR1 KO mice after morphine and buprenorphine administration, but not after systemic delivery of fentanyl or DAMGO. These variances indicate K_{ATP} channel subunits may be differentially modulated in areas of the nervous system, as changes in locomotion was not affected in the same degree by different classes of opioids compared to

antinociception. The degree of activation of K_{ATP} channels subtypes may be agonist and location dependent due to differences in opioid receptor coupling with the different downstream targets and protein-protein interactions [63], or differences in cellular metabolism in different nervous system areas (e.g. ATP levels). We are confident that the contribution of SUR1 deficiency in the spinal cord as it relates to drug seeking behavior appears to be minimal, as intrathecal administration of AAV9-Cre viral vectors to reduce SUR1 expression in flox mice does not appear to affect locomotion significantly, but does impact morphine antinociception and tolerance [16]. Although full dose response curves were not performed for buprenorphine, DAMGO, or fentanyl in this study, previous studies in global knockout models indicate that the changes in nociceptive or locomotor effects are minimal across higher doses [28,64,65]. Together, these data suggest K_{ATP} channel expression in the spinal cord and DRG contribute to antinociception, while K_{ATP} channel expression in higher order brain areas must be responsible for drug-induced hyperlocomotion.

The buprenorphine and fentanyl induced potassium flux seen in the DRG and spinal dorsal horn was significantly attenuated in SUR1 KO mice. This is in contrast to morphine, where changes in potassium flux were only seen in the DRG of SUR1 KO mice. After DAMGO treatment, a significant decrease in potassium flux was not found in either DRG or spinal cord dorsal horn cells. This data are largely in agreement with the evoked mechanical and thermal withdrawal behavioral data indicating that a global loss of SUR1 subtype K_{ATP} channels may have direct effects on pain signaling, as SUR1 KO mice have slightly altered mechanical thresholds compared to WT mice [11]. It is also possible the administration of morphine, buprenorphine, or fentanyl also have indirect actions stimulating descending inhibitory pathways that eventually lead to K_{ATP} channel activation. Although morphine and buprenorphine are considered prototypical μ opioid agonists, they also display activity at kappa and delta opioid receptors. Comparing the morphine and buprenorphine results with DAMGO suggests other non-opioid mechanisms may also play a role in K_{ATP} channel-induced antinociception. Experiments using K_{ATP} channel agonists in cells or animals lacking the MOR could give an indication if these effects are exclusive to activation of one opioid receptor subtype over another, or recruitment of additional receptors.

In conclusion, the results of the present study suggest SUR1-subtype K_{ATP} channels are important in mediating the analgesic effects of several classes of opioids, while mediating some effects of drug seeking behaviors. Ongoing studies should focus on identifying the nervous system regions and neural circuits that mediate these effects in the brain and spinal cord, and if other K_{ATP} channel subtypes co-facilitate these effects.

Author contributions

Sakamaki, G; Writing - review & editing, Writing - original draft, Formal analysis, Data curation. Johnson K; Writing - review & editing, Methodology, Writing - original draft, Data curation. Mensinger M: Investigation, Data curation. Hmu E: Data curation. Klein AH; Conceptualization, Resources, Funding acquisition, Supervision, Writing - review & editing. All the authors have made a substantial contribution for the conception, design, and drafting the article. All the authors have approved the version to be submitted.

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Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bbr.2021.113467.

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