The Journal of Headache and Pain

RESEARCH

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Enhancement of the endocannabinoid system through monoacylglycerol lipase inhibition relieves migraine-associated pain in mice

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Abstract

Background Migraine affects over 1 billion people worldwide and is a leading cause of disability. Targeting the cannabinoid system offers a promising approach for pain and migraine relief. This study evaluated a novel monoacylglycerol lipase (MAGL) inhibitor to prolong endocannabinoid action in acute and chronic mouse models of migraine. It also examined MAGL and cannabinoid receptor 1 (CB_1) mRNA expression in key head pain-processing regions.

Methods C57BL6/J male and female mice received the human migraine trigger nitroglycerin (NTG) acutely or every other day for 9 days. Allodynia was assessed by von Frey hair stimulation of the periorbital area. A single dose of MAGL inhibitor (ABD-1970) was tested in acute and chronic NTG models. Additionally, ABD-1970 was given daily for 5 days to assess tolerance. In situ hybridization measured transcript expression of MAGL, CB_1, and neuronal marker Rbfox3 in trigeminal ganglia (TG) and trigeminal nucleus caudalis (TNC).

Results A single injection of ABD-1970 blocked cephalic allodynia induced by acute NTG. ABD-1970 also blocked chronic allodynia established by chronic intermittent NTG. Repeated administration did not induce tolerance, and ABD-1970 continued to block NTG-induced allodynia after 5 days of administration. There was high expression of MAGL and CB_1 in the TG and TNC, present in Rbfox3 positive and negative cells.

Conclusion MAGL inhibitor effectively blocked acute and chronic migraine-associated pain, likely through prolonged endocannabinoid action. This effect may be mediated through action at peripheral or central sites considering the high MAGL and CB_1 expression in the TG and TNC, respectively. The endocannabinoid system appears to modulate migraine mechanisms, and MAGL may be a promising target for this disorder.

Keywords Headache, Endocannabinoid, Trigeminovascular pain, RNAScope

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Introduction

Headache disorders ranked as the third leading cause of years lived with disability in 2019, with migraine accounting for 88.2% of this burden [1]. Migraine affects ~ 15% of the global population, impacting roughly 1 in 6 Americans [2, 3]. The most disabling form of migraine is chronic migraine, and ~ 3% of patients transition from episodic to chronic migraine yearly [4]. Chronic migraine is defined as ≥ 15 headache days/month lasting at least 3 months [5, 6], and affects 1–2% of the population. A systemic review from 2024 found that disability associated with migraine almost doubled between 2005 and 2018, even though prevalence remained stable [7]. There is an enormous unmet need for millions of migraine sufferers for whom currently available therapies are only partially effective or poorly tolerated [8, 9].

The endocannabinoid system has emerged as a promising therapeutic target for headache disorders [10]. This system includes two primary endogenous ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which activate two main G-protein coupled receptors: cannabinoid receptor 1 (CB_1) and cannabinoid receptor 2 (CB_2) [11–13]. CB_1 receptors are primarily found on neurons within the central nervous system, while CB_2 receptors are predominantly located in the peripheral nervous system and on immune cells [11]. After their release, AEA is primarily degraded by fatty acid amide hydrolase (FAAH), and 2-AG by monoacylglycerol lipase (MAGL) [11].

Interest in the cannabinoids and endocannabinoids as a potential treatment strategy for migraine is growing. Approximately 36% of patients with migraine and headache have reported using exogenous cannabinoids to alleviate symptoms [14, 15]. In one study, medical marijuana was associated with a significant reduction in migraine frequency [16]. Chronic migraine is often linked to medication overuse headache, with positive correlations between increased use of triptans or opioids and increased headache days [17]. Notably, cannabis use was associated with a reduction in opioid use, as well as reduced medication use for migraine treatment [18]. These findings align with the clinical endocannabinoid deficiency (CECD) hypothesis, which suggests that reduced endocannabinoid tone in migraine patients may contribute to their pain and symptomatology [10]. Studies have shown that levels of 2-AG and AEA are significantly lower in chronic migraine patients than in controls [19, 20]. Interestingly, a negative correlation was reported between levels of calcitonin gene-related peptide (CGRP) or nitric oxide, two migraine triggers, and the level of AEA [20]. Furthermore, increased FAAH levels have been reported in the platelets of women with migraine without aura [21], and MAGL mRNA expression was found to be elevated in both episodic and chronic migraine patients [22].

Although exogenous cannabinoids may reduce migraine frequency, their effects are unpredictable and associated with adverse effects such as dizziness, dry mouth, nausea, cognitive impairment, psychosis, and abuse liability [14, 16, 23]. Given these limitations, cannabis' Schedule I status, and that cannabinoids are still illegal in many places, alternative strategies are needed. For example, a recent study showed that a peripherally restricted CB1 agonist was effective in a mouse model of migraine with limited tolerance [24]. Alternatively, enhancing endocannabinoid levels by inhibiting their degradation represents a promising approach. Acute FAAH inhibition produced anti-allodynic effects in an acute nitroglycerin (NTG) migraine model [25, 26]. Interestingly, in rat trigeminal ganglia, MAGL activity was approximately nine-fold higher than FAAH activity, suggesting that MAGL inhibition could be particularly effective [27]. Reversible (URB602) and irreversible (JZL184) MAGL inhibitors tested in mice, blocked hyperalgesia induced by NTG, a known human migraine trigger [28]. Moreover, a dual FAAH/MAGL inhibitor was effective in an acute NTG migraine model as well as blocking meningeal signaling from application of KCl, in a model of migraine aura [29–31].

Previous studies of irreversible MAGL inhibitors have shown efficacy in pain models such as traumatic nerve injury and chemotherapy-induced peripheral neuropathy [32]. However, centrally acting inhibitors led to unwanted side effects, including pharmacological tolerance through receptor desensitization, as well as physical dependence [32]. In migraine models, MAGL inhibitors have been tested in male animals and in acute migraine models, but not in female animals or in chronic states. Recently, a novel potent and selective MAGL inhibitor (ABD-1970, or Lu AG07779) was reported to produce analgesic effects without overt side effects [33]. Therefore, we aimed to investigate the therapeutic potential of MAGL inhibition using ABD-1970 in acute and chronic models of migraine in male and female mice.

Materials and methods

Animals

Experiments were performed on male and female C57BL6/J mice (Jackson Laboratories). Mice were group housed in a 12 h–12 h light-dark cycle, and lights turned on at 07:00 and turned off at 19:00. Food and water were available ad libitum. All animal experiments were performed according to the Association for Assessment and Accreditation of Laboratory Animal Care guidelines administered by the University of Illinois at Chicago and Washington University School of Medicine Animal Care Committees.

Drugs

ABD-1970 (or Lu AG07779, provided by Lundbeck) was dissolved in 0.5% methylcellulose in water and administered PO at 10 mg/kg dose. Olcegepant (BIBN 4096) was diluted in 0.9% saline and 1% DMSO and administered IP at 1 mg/kg dose (TOCRIS, Bristol, United Kingdom). ABD-1970 and olcegepant were injected 4 h prior to behavioral testing. Sumatriptan was diluted in 0.9% saline and administered IP at 0.6 mg/kg dose (Sandoz, NC, USA). The delta opioid receptor (DOR) agonist, SNC80 (Tocris Bioscience, Pittsburgh, PA) was dissolved in acidic 0.9% saline pH 5.5 and administered IP at 10 mg/kg dose. Sumatriptan and SNC80 were injected 45 min prior to behavioral testing. NTG (American Regent) was diluted to a 10 mg/kg dose with 0.9% saline. All drugs were made fresh on each test day and were administered in a volume of 10 ml/kg. Dose finding studies for ABD-1970 in mice found that 10 mg/kg effectively inhibited MAGL brain activity and increased 2-AG concentration [33]. Doses of olcegepant, sumatriptan, and SNC80 have previously shown efficacy in the NTG model and act as positive controls [34–37].

NTG-induced pain models

To measure cephalic mechanical responses, we used manual von Frey hair stimulation, and the up-and-down method (force ranging from 0.008 g to 2 g) as described previously [37, 38]. All animals were habituated for 2 days prior to first test day, and on each test day for 1 h prior to the first measurement. Mice were tested in 4 oz paper cups included in the testing apparatus during habituation. Mice were counterbalanced into groups following the first baseline test. All responses were conducted in a blinded fashion by 1–2 experimenters. A response was defined as shaking, ducking of head, or vigorous grooming of the cephalic region following stimulation.

For acute model, a single 10 mg/kg nitroglycerin (NTG) or vehicle saline dose was administered IP and tested 2 h later. For chronic model, NTG was administered on days 1, 3, 5, 7, and 9 [35]. For cephalic testing, thresholds were assessed on days 1, 5, and 9. At least 4 h were maintained between baseline and post-drug testing.

RNAScope fluorescent in-situ hybridization

RNAscope kit was purchased from Advanced Cell Diagnostics RNAScope Technology (ACD Bioscience). C57Bl6/J mice were anesthetized with isoflurane, and the brain and TG were collected fresh frozen with dry ice in cryoprotectant. Frozen tissue was cut on a cryostat at 10 µm, collected on slides, and processed per the manufacturer's protocol. The probes used were targeted against the mouse genes for CB_1 (*CNR1*), MAGL (*MGLL*) and NeuN (*RBFOX3*). All samples were observed under a Keyence BZ microscope with BZ-X800 analyzer software (Keyence). Quantification of RNAscope fluorescence was done with HALO image analysis (Indica Labs) for any expression indicating a possible cell from 20x images [39].

Quantification and statistical analysis

Results are presented as mean ± SEM. Statistical analyses were conducted using Graph-Pad Prism (version 10, GraphPad Software, Inc.). Data were analyzed using one-, or two-way analysis of variance followed by Holm-Sidak multiple comparison tests. A value of $p < 0.05$ was considered statistically significant unless otherwise noted. Detailed statistical analysis can be found in the Supplementary Statistics Table.

Results

In the current study, we focused on whether MAGL inhibitor ABD-1970 could block acute and chronic NTG-induced cephalic allodynia. Cephalic mechanical responses were measured in an equal number of male and female C57BL6/J mice/group at two time points (Fig. 1A): before any drug treatment (Baseline, Fig. 1B), and 2 h after NTG/Veh injection (Fig. 1C). The timing at which drug effects were tested was based on pharmacokinetic properties of each drug. ABD-1970 (10 mg/kg PO) or Veh (0.5% Methylcellulose PO) was administered 2 h prior to NTG (10 mg/kg IP) or Veh (saline). Positive controls included known migraine therapeutics: a gepant (olcegepant, a CGRP receptor antagonist) and a triptan (sumatriptan, 5-HT1B/D receptor agonist). The olcegepant (1 mg/kg IP in 1% DMSO and saline) group was administered drug 2 h before NTG/Veh, and the sumatriptan (0.6 mg/kg IP in saline) group 45 min after NTG. All groups started with a similar baseline cephalic mechanical threshold (Fig. 1B). A single injection of NTG produced a significant decrease in mechanical threshold (Fig. 1C, NTG-VEH). ABD-1970 blocked NTG-induced allodynia. This effect was comparable to the anti-allodynic effect of olcegepant and sumatriptan (Fig. 1C). These results establish that acute migraine-associated pain can be blocked by MAGL inhibition.

We next determined if chronic allodynia induced by chronic intermittent NTG was sensitive to treatment with a MAGL inhibitor. Mice were treated with vehicle or NTG (10 mg/kg IP) every other day for 9 days. Cephalic thresholds were determined on days 1, 5, and 9 (Fig. 2A). NTG produced a significant and sustained cephalic allodynia (Fig. 2B). Drugs were tested on day 10, ~ 20 h after the final NTG/Veh injection. Mice treated with NTG continued to show a significant decrease in cephalic mechanical threshold compared to vehicle controls (Fig. 2B day 10, Fig. 2C NTG/Veh). After baseline on day 10, ABD-1970 (10 mg/kg PO), Veh (0.5% Methylcellulose PO), or olcegepant (1 mg/kg IP in 1% DMSO and saline)

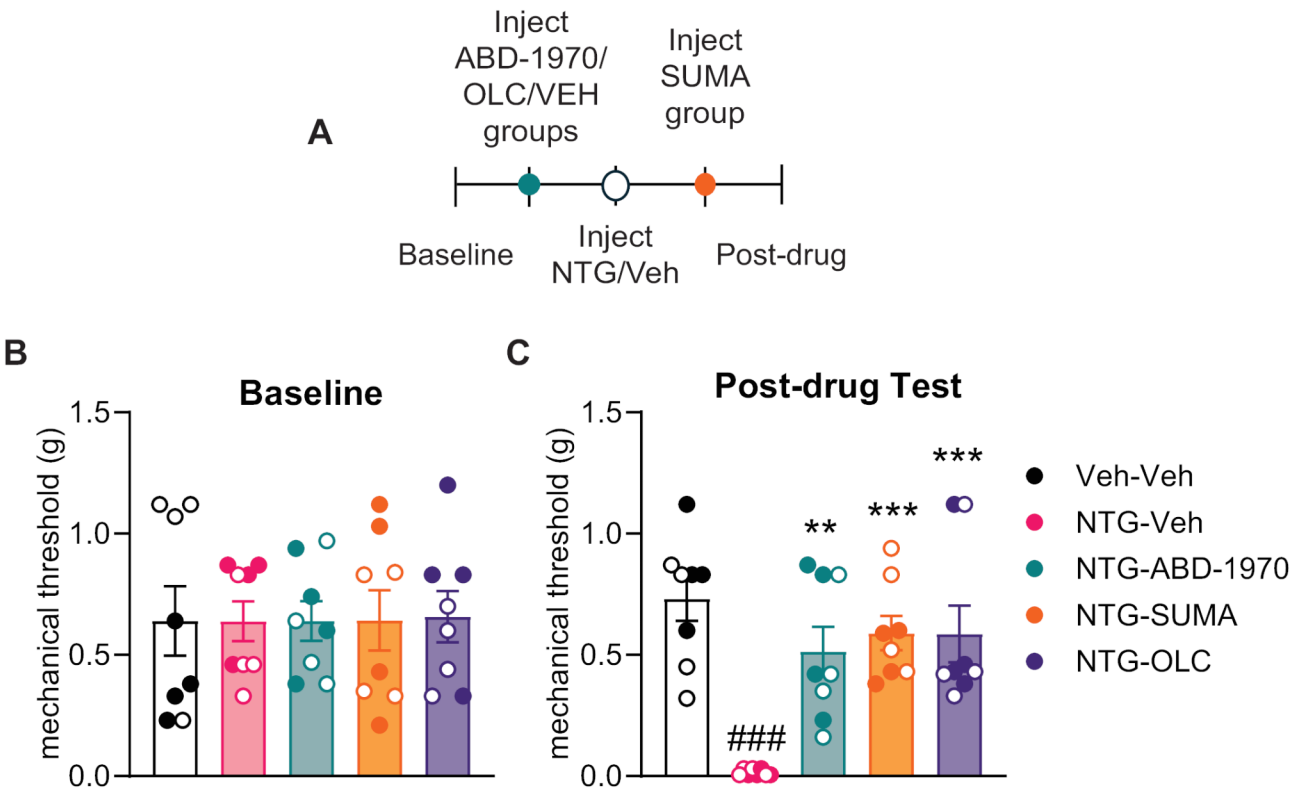

Fig. 1 Acute nitroglycerin (NTG)-induced cephalic allodynia is blocked by acute MAGL inhibition in male and female C57BL6/J mice. (**A**) Schematic of paradigm includes measuring baseline cephalic thresholds, injecting ABD-1970 (10 mg/kg PO), methylcellulose vehicle control (PO), or olcegepant (1 mg/kg IP) 4 h before von Frey testing (teal circle), injecting nitroglycerin (10 mg/kg IP) or saline vehicle 2 h before testing (open circle), and injecting sumatriptan (0.6 mg/kg IP) 45 min before testing (filled in orange circle). (**B**) Basal responses and (**C**) post-drug test responses are shown ($n = 8$/group). 1-way ANOVA analysis, significant effect of treatment ($p < 0.001$). Holm-Sidak multiple comparisons: $^{**}p < 0.01$, $^{***}p < 0.001$ relative to NTG-Veh, $^{\#\#\#}p < 0.001$ relative to Veh-Veh. Female animals indicated with open circles. All data are presented as mean ± SEM

was administered and tested 4 h later. In addition, the delta opioid receptor (DOR) has emerged as a promising therapeutic for headache disorders [37, 40]. Therefore, we also included the DOR agonist SNC80 (10 mg/kg IP) which we administered 45 min before testing. ABD-1970 completely inhibited chronic NTG-induced cephalic allodynia, similar to olcegepant and SNC80 (Fig. 2C).

Previous rodent studies have shown the development of pharmacological tolerance after repeated high doses of MAGL inhibitors [41, 42]. Thus, we next investigated if repeated administration of ABD-1970 would lead to tolerance or decreased analgesic effectiveness in the acute NTG model. Mice were injected with ABD-1970 (10 mg/kg PO) or Veh (0.5% Methylcellulose PO) once daily for 4 days (Fig. 3A). On day 5, mice underwent the acute NTG model. On this day, ABD-1970 mice received a final dose of drug 2 h prior to NTG treatment. The positive control, olcegepant (1 mg/kg IP in 1% DMSO and saline), was also administered at this time point on test day. The other positive control, sumatriptan (0.6 mg/kg IP in saline) was administered 45 min after NTG treatment on test day. Chronic administration of ABD-1970 did not change baseline cephalic mechanical thresholds (Fig. 3B, day 5). Acute NTG produced a significant decrease in mechanical threshold that was blocked by ABD-1970. This effect was comparable to the anti-allodynic effect of olcegepant and sumatriptan (Fig. 3C). Together this data indicates that the MAGL inhibitor ABD-1970 did not induce tolerance and continued to effectively block migraine-associated pain.

We used in situ hybridization to examine the expression and co-expression of MAGL, CB_1, and NeuN (Rbfox3) mRNA in the TG and TNC. These regions are involved in head and meningeal innervation and regulate head pain processing. Representative images show the distribution of MAGL (yellow), CB_1 (teal), and Rbfox3 (magenta) transcripts in these regions (Fig. 4A). To quantify expression, we measured the percentage of cells in the TG (Fig. 4B) and TNC (Fig. 4C) that expressed each transcript. In the TG (peripheral nervous system-PNS), ~45% of cells expressed the neuronal marker Rbfox3, ~50% expressed MAGL, and ~25% expressed CB_1 transcript (Fig. 4B). In the TNC, ~50% of cells were Rbfox3 positive, ~75% were MAGL positive, and ~40% were CB_1 positive (Fig. 4C).

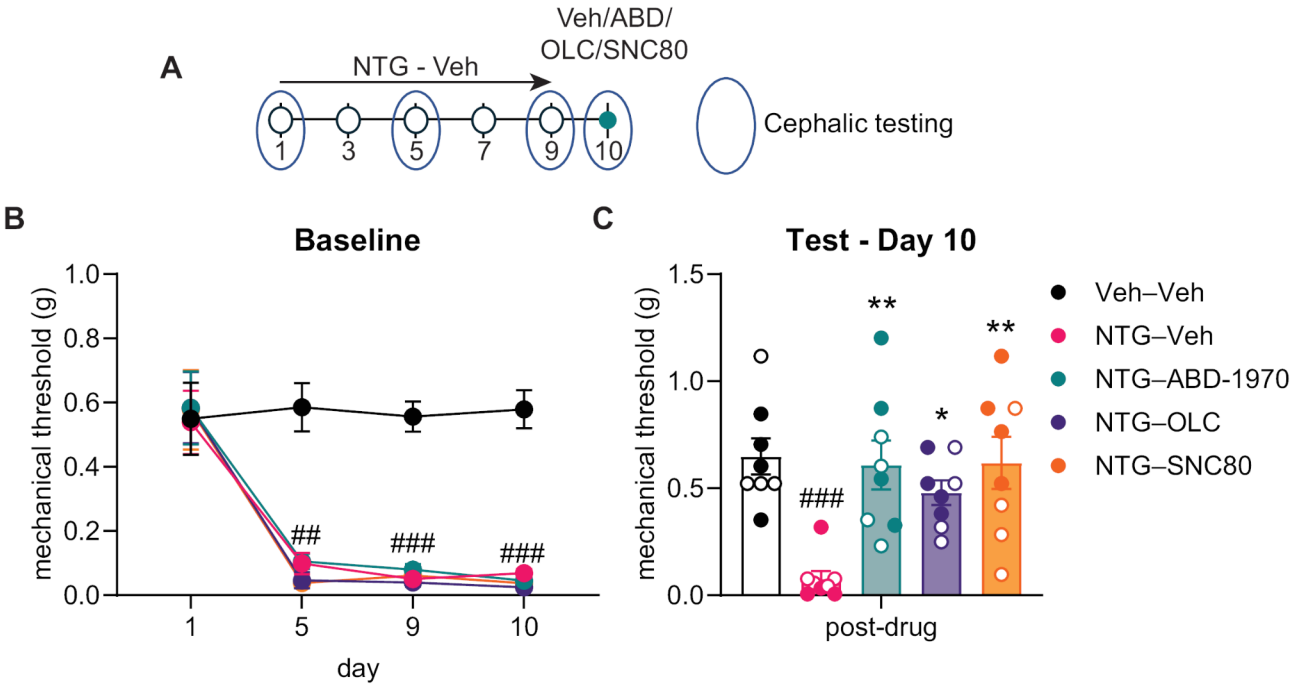

Fig. 2 Chronic NTG-induced cephalic allodynia is blocked by acute MAGL inhibition in male and female C57BL6/J mice. (**A**) Schematic of paradigm includes animals receiving vehicle or NTG (10 mg/kg IP) every other day for 9 days (open circle), with baseline mechanical responses measured on days 1, 5, 9, and 10. Animals were injected with Veh, ABD-1970, olcegepant, or SNC80 and tested (filled in teal circle). (**B**) Chronic NTG led to a decrease in mechanical threshold that lasted at least ~24–48 h after last injection. ($n = 8$/group). 2-way repeated measures ANOVA analysis, significant effect of time, treatment, and interaction ($p < 0.001$). Holm-Sidak multiple comparisons: ##$p < 0.01$, ###$p < 0.001$ relative to Veh-Veh. (**C**) Post-drug responses after treatment with ABD-1970 (10 mg/kg PO), methylcellulose vehicle control (PO) or olcegepant (1 mg/kg IP) 4 h before testing, and DOR agonist SNC80 (10 mg/kg IP) or saline vehicle (IP) 45 min before testing post-drug thresholds ($n = 8$/group). 1-way ANOVA analysis, significant effect of treatment ($p < 0.001$). Holm-Sidak multiple comparisons: *$p < 0.05$, **$p < 0.01$ relative to NTG-Veh, ###$p < 0.001$ relative to Veh-Veh. Female animals indicated with open circles. All data are presented as mean ± SEM

Next, we analyzed the co-expression patterns and distribution of cells which express MAGL, CB_1, MAGL + CB_1, or neither transcript in neuronal (Rbfox+) versus non-neuronal (Rbfox-) cells. In the TG (PNS), ~30% of neuronal cells expressed MAGL, ~10% CB_1, ~45% MAGL + CB_1, and ~16% expressed neither transcript (Fig. 4D). Of Rbfox3 negative cells (non-neuronal), ~30% of cells expressed MAGL, ~1% CB_1, 2% MAGL + CB_1, and 67% neither transcript (Fig. 4E). In the TNC (CNS), ~38% of neuronal cells expressed MAGL, ~8% CB_1, ~50% MAGL + CB_1, and ~6% neither transcript (Fig. 4F). Among non-neuronal cells, ~48% had MAGL transcript, ~8% CB_1, ~20% MAGL + CB_1, and ~24% neither transcript (Fig. 4G). Overall, these results suggest that CB_1 and MAGL are expressed on both neurons and non-neuronal cells. MAGL was generally more prevalent than CB_1, which corresponded to a higher percentage of CB_1 + cells also expressing MAGL transcript.

Discussion

The current study suggests that enhancing the endocannabinoid system may modulate acute and chronic migraine-associated pain. We found that a single injection of the human migraine trigger, NTG, led to cephalic allodynia, which was blocked by the novel MAGL inhibitor ABD-1970. Strikingly, ABD-1970 also blocked chronic migraine-associated pain induced by chronic intermittent administration of NTG. ABD-1970 had a similar effect size to other known and experimental migraine treatment strategies including a triptan, CGRP receptor antagonist, and DOR agonist. Importantly, chronic treatment with ABD-1970 did not produce tolerance. We also found substantial expression of MAGL and the CB_1 receptor transcripts in head pain processing areas. These results suggest that MAGL inhibitors could act peripherally or centrally and on neuronal or non-neuronal cells to alleviate headache.

Endocannabinoids, AEA and 2-AG are expressed in the trigeminal ganglia (TG) and trigeminal nucleus caudalis (TNC) [43]. There is little expression of CB_2 receptor in these regions [44–46]. Previous reports indicate CB_1 receptor mRNA in rats was expressed in approximately 30% of TG neurons, and immunohistochemistry showed ~15% pixel/micron^2 neuronal CB_1 in the TG and ~10% pixel/micron^2 neuronal CB_1 in the TNC [44–47]. MAGL is present in the TG and TNC, although mRNA expression using in situ hybridization in these areas has not been well characterized previously [27, 28,

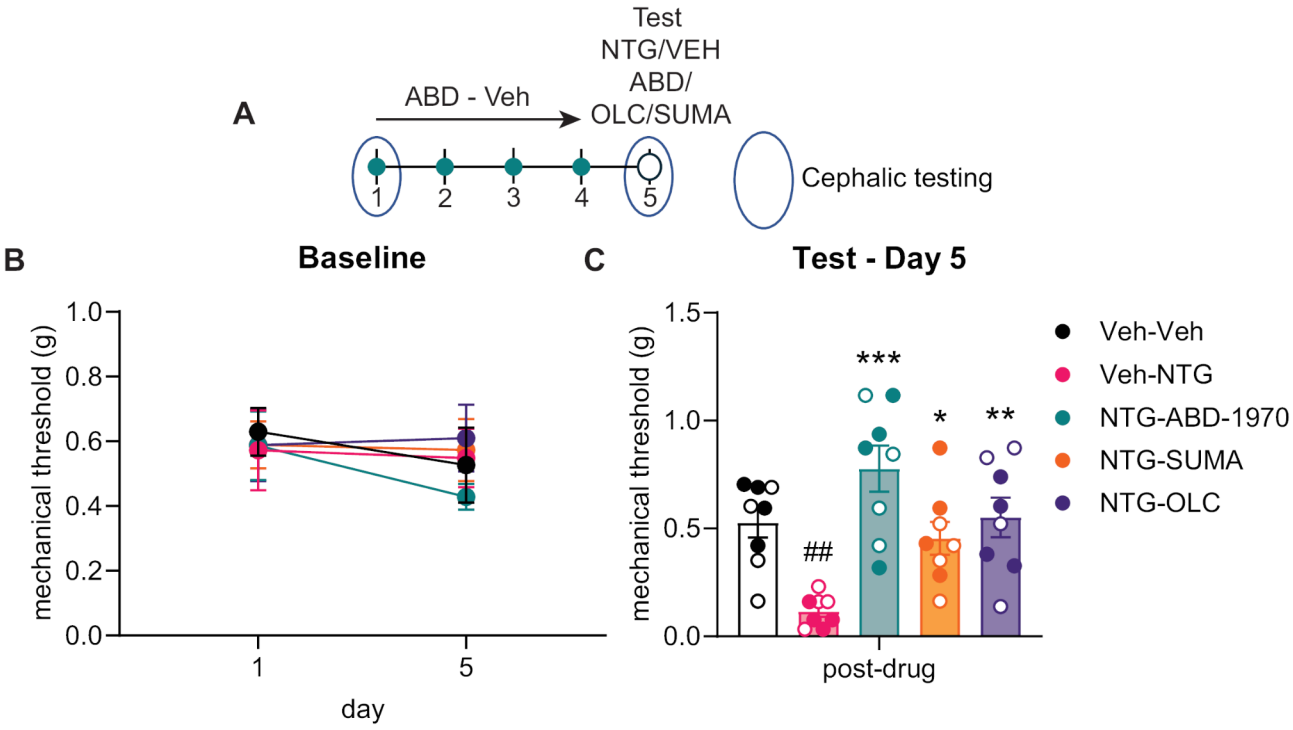

Fig. 3 Chronic MAGL inhibition caused no change in baseline mechanical threshold and continued to block acute migraine-associated pain in male and female C57BL6/J mice. (**A**) Schematic of paradigm– mice were treated with chronic MAGL inhibitor (ABD-1970 (10 mg/kg, 5x QD, PO) or methylcellulose vehicle control (PO)) every day for four days (filled in teal circle). On day 5 baselines were taken, and then animals were injected with Veh, ABD-1970, sumatriptan, or olcegepant before taking post-drug thresholds (open circle). (**B**) On day 5, baselines were taken ($n=8$/group). 2-way repeated measures ANOVA analysis showed no significant effect of time ($p=0.8225$), treatment ($p=0.2736$) or interaction ($p=0.2826$) after chronic MAGL inhibition. (**C**) Then mice were injected with ABD-1970 (10 mg/kg PO), methylcellulose vehicle control (PO) or olcegepant (1 mg/kg IP) 4 h before testing. NTG or saline vehicle (10 mg/kg IP) was administered 2 h before testing, and sumatriptan (0.6 mg/kg IP) 45 min before testing post-drug thresholds ($n=8$/group). 1-way ANOVA analysis, significant effect of treatment ($p<0.001$). Holm-Sidak multiple comparisons: *$p<0.05$, **$p<0.01$, ***$p<0.001$ relative to NTG-Veh, ##$p<0.01$ relative to Veh-Veh. Female animals indicated with open circles. All data are presented as mean ± SEM

48]. Aligned with these findings, here we described the transcript expression of CB_1 and MAGL in the trigeminal complex and further characterized neuronal versus non-neuronal expression. We found substantial MAGL expression in peripheral TG (~50%), and the majority of neurons in this region expressed MAGL (~75%) and ~30% on non-neuronal cells. In comparison, only ~25% of cells in the TG expressed CB_1 receptor, and the majority of this expression was neuronal. In the TNC, ~75% of cells expressed MAGL, and almost 90% of neurons and 70% of non-neurons expressed this transcript. Comparatively, ~40% of cells in this brain region expressed CB_1, and approximately 60% of neurons and 30% of non-neuronal cells expressed this transcript. Interestingly, on neurons there was a high co-expression of MAGL + CB_1 in both TG and TNC. These results suggest that the endocannabinoid system is well placed in the trigeminal system to potentially serve as an endogenous facilitator of headache [28, 49].

Downregulation of endocannabinoids have been observed in migraine patients [20], and this deficiency has been postulated as a cause for migraine [49]. This notion may explain effects of NTG, and acute NTG treatment was shown to increase MAGL and FAAH levels in several brain regions [48, 50]. MAGL was also found to be upregulated in the periaqueductal grey in a cortical spreading depression model of migraine [31]. Previously, a DAGL inhibitor reduced 2-AG production and signaling in the brain which induced headache-like pain in rats, further supporting the role of endocannabinoid downregulation in migraine pathophysiology [51]. We found that inhibiting MAGL activity before NTG treatment inhibited acute cephalic allodynia. Importantly ABD-1970 inhibited established chronic migraine-associated pain even when NTG was no longer present. This finding is clinically important as chronic migraine is highly disabling [52] and many patients seek treatment only once migraine has shifted from an episodic to chronic condition.

Enhancement of the endocannabinoid system appears to be a promising approach for the treatment of headache disorders. The MAGL inhibitor, MJN110, was found to prevent and reverse allodynia induced by cortical spreading depression [31]. Furthermore, the same inhibitor

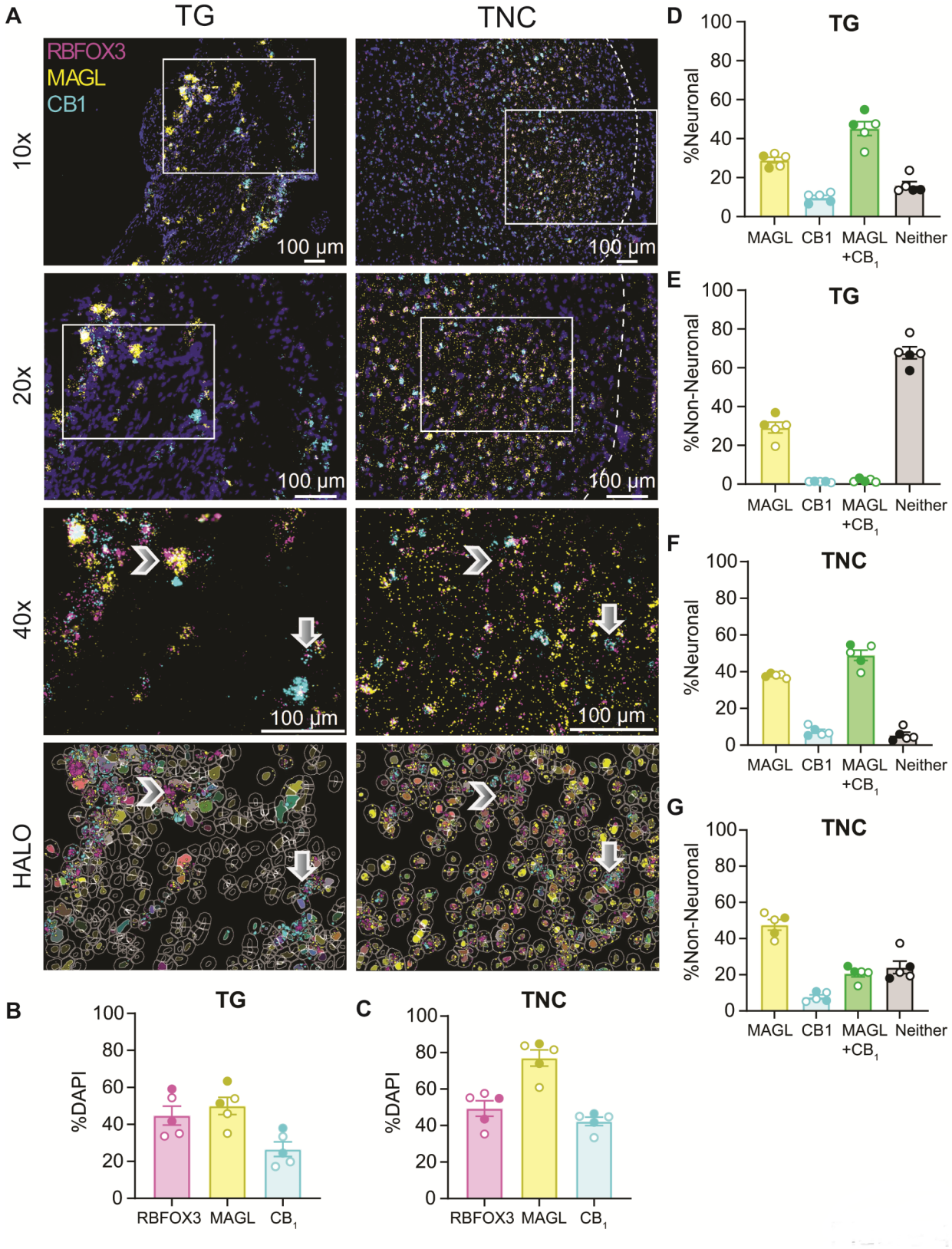

Fig. 4 (See legend on next page.)

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Fig. 4 MAGL is highly expressed in the TG and TNC, with higher expression in Rbfox3/NeuN expressing cells. (**A**) Representative images of fluorescent in situ hybridization. Chevron indicates a cell expressing MAGL and Rbfox3, arrow indicates a cell expressing CB_1 and Rbfox3 transcripts. Percentage of total cells expressing transcripts stained with DAPI in (**B**) TG and (**C**) TNC. Percentage of total Rbfox3 + cells expressing MAGL and/or CB_1 transcript in the (**C**) TG and (**E**) TNC. Percentage of total Rbfox3 negative cells expressing MAGL and/or CB_1 transcript in the (**D**) TG and (**F**) TNC. Data are the average from 5 mice, at least four 20x images/mouse were quantified using HALO software. Data represented as mean ± SEM. Females are represented with clear symbols

also reduced cephalic allodynia in a model of post-traumatic headache [43]. FAAH has also been examined for migraine, and FAAH knockout mice showed decreased responses to NTG and FAAH antagonist blocked NTG-induced allodynia in wildtype mice [26]. In addition, dual inhibition of FAAH and MAGL by JZL195 inhibited pain-like behaviors induced by NTG [29], and another dual inhibitor, AKU-005, decreased trigeminal nerve excitation induced by KCl [30]. Along with the results presented in this study, this work provide compelling evidence for targeting endocannabinoid degradation enzymes for migraine.

The exact mechanism for cannabinoid pain relief is still unknown. One potential mechanism is endocannabinoid-induced reduction of meningeal mast cell degranulation [53]. Additionally, inhibitory cannabinoid action can reduce release of pro-migraine molecules such as CGRP [46]. Acute and chronic CGRP targeting therapies like gepants and antibodies show a reduction in migraine pain and frequency [3]. However, they are not effective for all patients and provide incomplete symptom relief [54]. Targeting the endocannabinoid system would increase the migraine pharmacological tool box, and may alleviate migraine that is non-responsive to existing therapies.

Notably, pharmacological treatment can have significant limitations including off target effects, stability, and tolerance. Critically, in comparison to previously tested MAGL inhibitors [41, 42], as well as clinically used opioids, here we demonstrated that repeated ABD-1970 treatment did not lead to tolerance development, and ABD-1970 maintained efficacy in blocking NTG-induced cephalic allodynia. Several CB1 agonists, as well as the brain-penetrant MAGL inhibitor JZL184 produced tolerance after chronic administration [42, 55, 56]. A potential explanation for why ABD-1970 did not induce tolerance while JZL184 did is the difference in dosing and duration of administration. JZL184 was administered at 40 mg/kg for 6 days, whereas we tested ABD-1970 at 10 mg/kg for 4 days [42]. Further, ABD-1970 may induce a protein conformation state that favors decreased desensitization/downregulation or increased resensitization. In comparison to exogenous CB_1 agonists, MAGL inhibitors enhance localized endocannabinoid release in the body and are less likely to result in super-activation of receptor signaling. Tolerance to classic preventative treatments occurs in approximately 1–8% of patients [57]. In the clinic it can be difficult to distinguish between tolerance and medication overuse headache (MOH), a paradoxical increase in headache frequency and severity after chronic drug use. Approximately 12.6% of migraine patients experience MOH [58]. CGRP therapeutics are novel treatments for migraine, with limited MOH [59] and tolerance. However, a recent meta-analysis identified a "weaning-off" effect and decreased efficacy of CGRP mAbs in chronic migraine patients after multiple injections [60].

Several limitations of our study should be acknowledged. Testing ABD-1970 in other migraine models such as CGRP or PACAP-induced headache models [39] would better characterize the molecular mechanisms regulating endocannabinoid action on migraine. In the current study we focused on cephalic allodynia; however, allodynia can spread to extracephalic regions, and peripheral responses or other non-evoked endpoints were not tested. ABD-1970 is brain penetrant, and it is not clear if restricting MAGL inhibitors to the periphery would be sufficient to inhibit migraine-associated pain. We recently showed that a peripherally restricted CB1 agonist was effective in the NTG model [24], and future studies will determine if peripheral MAGL inhibitors also show efficacy for migraine. Increased signaling at the CB1 receptor after ABD-1970 was assumed with the increase in MAGL activity and 2-AG brain concentration previously identified [33], but a future experiment examining the effect of a CB_1 antagonist on ABD-1970 would better prove its action at this receptor. Additionally, the expression of MAGL and CB_1 was limited to transcript analysis, due to a dearth of specific antibodies targeting these proteins. The in situ hybridization approach we used could not provide information on cellular characteristics beyond neuronal vs. non-neuronal cells. Future studies could for example assess MAGL and CB1 expression in peptidergic or non-peptidergic cells in the TG. Furthermore, transcript expression does not necessarily result in the translation to protein, and protein may be expressed in efferents rather than locally. An additional future direction would be to analyze how transcript expression can be altered after acute or chronic NTG. Male and female animals were tested in this study, and no significant differences were identified, but our experiments were underpowered. Therefore, replication in larger samples would establish any potential sex differences.

Conclusion

Our findings indicate that inhibiting MAGL activity blocks the development of cephalic mechanical allodynia following acute NTG treatment, similar to clinically used migraine treatment strategies. Enhancing the endocannabinoid system by blocking MAGL, strikingly also improved cephalic mechanical allodynia related to chronic migraine-associated pain. Critically, chronic treatment with ABD-1970 showed no signs of tolerance development. This data suggests that MAGL inhibitors may be safe and efficient in modulating the development and maintenance of migraine. Promisingly, a MAGL inhibitor Lu AG06466 progressed to phase 2 clinical trials for Tourette's syndrome and has demonstrated safety in these studies, suggesting that MAGL inhibitors like ABD-1970 may be well tolerated for the treatment of migraine [61, 62].

Abbreviations

MAGL	Monoacylglycerol lipase
MAGLi	Monoacylglycerol lipase inhibitor
NTG	Nitroglycerin
CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
2-AG	2-Arachidonoylglycerol
AEA	N-arachidonoylethanolamine, anandamide
TG	Trigeminal ganglia
TNC	Trigeminal nucleus caudalis
IP	Intraperitoneal
SC	Subcutaneous
PO	Per os/gavage
FAAH	Fatty acid amide hydrolase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s10194-025-02029-9.

Supplementary Material 1

Author contributions

Conceptualization: EM, FG, AA, AP; Data Curation: EM, YA, KS; Formal Analysis: EM, YA, KS, AP; Investigation: EM, YA, KS, AP; Methodology: EM, YA, KS, FG, AA, AP; Project Administration: EM, AP; Supervision: AP; Funding Acquisition: AP; Validation: EM, YA, KS; Visualization: EM, YA; Writing– first draft: EM; Writing–review & editing: EM, YA, FG, AA, AP.

Funding

H. Lundbeck A/S.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All animal experiments were performed according to the Association for Assessment and Accreditation of Laboratory Animal Care guidelines administered by the University of Illinois at Chicago and Washington University School of Medicine Animal Care Committees.

Consent for publication

Not applicable.

Competing interests

Two of the authors (FG and AAA) are employees of Lundbeck. However, they did not collect or analyze data nor did they influence it in any way.

Received: 19 February 2025 / Accepted: 7 April 2025
Published online: 18 April 2025

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