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The effects of acute and preventive migraine therapies in a mouse model of chronic migraine

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Abstract

Background—The development of novel migraine therapies has been slow, in part because of the small number of clinically relevant animal models. We have recently developed a new mouse model of chronic migraine using chronic intermittent nitroglycerin, a known human migraine trigger. The objective of this study was to validate this model by testing known and potential migraine-preventive treatments.

Methods—Migraine therapies were administered to male and female mice for 11 days. On day 3, mice were tested with nitroglycerin every second day for nine days. Basal and nitroglycerin-evoked mechanical hypersensitivity was evaluated using von Frey filaments.

Results—Chronic intermittent nitroglycerin produced acute hyperalgesia with each administration, and progressive and sustained basal hypersensitivity. The established preventive migraine therapy propranolol effectively blocked the development of acute and chronic nitroglycerin-induced hyperalgesia, while valproate had no effect. Potential migraine-preventive therapies were also tested: Amiloride inhibited nitroglycerin-induced acute and chronic hyperalgesia; while memantine was ineffective. We also tested the acute migraine therapy sumatriptan, which did not alter nitroglycerin-induced hyperalgesia, but instead resulted in acute and chronic hyperalgesia similar to that observed following nitroglycerin administration.

Conclusions—This study establishes the chronic nitroglycerin model as an additional screening tool to test novel migraine-preventive therapies.

Keywords

Allodynia; prophylactic; pain; triptan

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Introduction

Migraine is a complex neurological disease that affects hundreds of millions of individuals worldwide. Migraine commonly progresses from an episodic to a chronic condition; this transformation occurs at an overall rate of 2.5% of patients each year, with a particularly high risk associated with overuse of acute medications (1,2). Chronic migraine is classified as headache on 15 or more days per month for more than three months, with at least eight of those days including symptoms that are consistent with the features of a migraine attack (3). In the United States, approximately 3 million people experience chronic migraine, and for a significant percentage of patients migraine results in chronic disability, costing more than \$15 billion/year in lost productivity (1,4,5). Despite the extraordinarily high prevalence of migraine, therapeutic strategies are limited, and less than 50% of chronic migraine patients are satisfied with their treatment (1). There continue to be millions of individuals for whom currently available migraine therapies are either ineffective or poorly tolerated (1,4).

A significant obstacle to the identification of new migraine therapies has been the difficulty in modeling this disorder in animals. One approach to modeling acute migraine is the quantification of increased sensory sensitivity in response to known migraine triggers. Nitroglycerin (NTG) reliably triggers headache in normal individuals (6,7); and NTG-evoked migraine is a commonly used experimental paradigm in humans (for review, see Olesen (7,8)). NTG-evoked hyperalgesia in rodents has been developed as a model for sensory hypersensitivity associated with migraine (9,10). Acute NTG produces thermal and mechanical hyperalgesia in mice that is reversed by the antimigraine therapies sumatriptan (SUMA) (9), and a calcitonin gene-related peptide (CGRP) receptor antagonist (11). In addition, NTG-evoked hyperalgesia is exacerbated in a transgenic mouse expressing the human migraine gene, casein kinase 1 delta (12). Further, NTG has also been shown to produce photophobia (10), and altered meningeal blood flow in mice (10,13). Taken together, these results indicate that the effects of NTG may effectively model migraine-like symptoms in rodents (14).

In order to better understand chronic migraine, we have recently modified the acute NTG model to examine the progression of migraine from an acute to chronic state (15,16). The administration of NTG every second day over nine days produces two distinct pain states—acute hyperalgesia following each injection of NTG, and a basal hypersensitivity that progressively develops over time. We showed previously that the acute migraine therapy SUMA could block the acute NTG-evoked hyperalgesia but had no effect on the basal hypersensitivity (15,16). In contrast, the migraine preventive topiramate (an anticonvulsant) could block both the development of chronic sensitivity and acute hyperalgesia (15). We hypothesize that the observed basal hypersensitivity following chronic NTG treatment models the progression of migraine from an episodic to a chronic disorder; and therefore chronic treatments that block the development of this hypersensitivity could predict the efficacy of migraine-preventive therapies. The objective of this study was to further characterize this mouse model of chronic migraine by testing known and novel migraine-preventive therapies. As a comparison, we also tested chronic SUMA, which is not considered to have preventive properties, and on the contrary may result in medication-

overuse headache. Our results indicate that this model of chronic migraine can act as a predictive tool to screen novel preventive therapies.

Methods

Animals

Subjects were male and female C57BL6/J mice (Jackson Laboratories, Bar Harbor, ME, USA), weighing 20–30 g. Animals were group housed in a 14–10 light-dark cycle, where the lights were turned on at six hours and turned off at 20 hours. Food and water were available ad libitum. All experimental procedures were approved by the University of Illinois at Chicago Office of Animal Care and Institutional Biosafety Committee, in accordance with Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) guidelines and the Animal Care Policies of the University of Illinois at Chicago. Animals were weighed daily during treatment, and no adverse effects of treatment were observed. All results are reported according to Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Drug administration

NTG was prepared from a stock solution of 5.0 mg/ml NTG in 30% alcohol, 30% propylene glycol and water (American Regent, Shirely, NY, USA). NTG was freshly diluted in 0.9% saline to a dose of 10 mg/kg. The vehicle (VEH) used in these experiments was 0.9% saline. We found previously that there was no significant difference in mechanical thresholds between 0.9% saline and the solution in which NTG was dissolved (6% propylene glycol, 6% alcohol, 0.9% saline) in an acute experiment (15). Animals were treated for a total of 11 days, during which an intraperitoneal (ip) injection of preventive drug or VEH was administered daily. On days 3,5,7,9 and 11 mice received an injection of NTG or VEH immediately following testing for basal mechanical sensitivity. The treatment of the mice on testing days was as follows: injection of preventive therapy, onto the test rack for habituation, 15–20 minutes later measurement of baseline mechanical thresholds, administration of NTG (10 mg/kg, 30–45 minutes post-preventive), and at two hours post-NTG animals were tested again for mechanical responses. All injections were administered as a 10 ml/kg volume. To test medication-overuse headache, SUMA was administered similar to the preventive therapy paradigm. Doses of the drugs tested were as follows: propranolol (PROP) 20 mg/kg, valproate (VA) 25 and 200 mg/kg, amiloride (AMIL) 10 mg/kg, memantine (MEM) 3 mg/kg, and SUMA 0.6 mg/kg.

Sensory sensitivity testing

All animal testing occurred in low-light conditions, between eight and 15 hours. To determine mechanical responses, the threshold for responses to punctate mechanical stimuli (mechanical hyperalgesia) was tested according to the up-and-down method. In brief, the plantar surface of the animal hindpaw was stimulated with a series of eight von Frey filaments (bending force ranging from 0.008 to 2 g). A response was defined as a lifting or shaking of the paw upon stimulation. The first filament tested was 0.4 g. In the absence of a response, a heavier filament (up) was tried, and in the presence of a response, a lighter filament (down) was tested. This pattern was followed for a maximum of four filaments

following the first response. For all experiments, groups were counterbalanced based on their naïve baselines (basal responses taken on day 1).

Statistical analysis

Data are expressed as mean $\pm$ s.e.m. All animals tested were included in the analysis. All statistical analyses were performed by SigmaStat software, and graphs were generated using GraphPad Prism. For all experiments a two-way repeated-measures analysis of variance (ANOVA) was performed, with drug and time as factors. When a significant interaction occurred, subsequent Holm-Sidak post-hoc analysis was performed. In this case, all groups were compared to responses on day 1, and to the vehicle-vehicle group. A significance level of $p < 0.05$ was used.

Results

For all experiments, mice were treated once daily with the test drug for 11 days. On days 3, 5, 7, 9, and 11, mice were tested for basal mechanical responses following which they were injected with NTG, and post-treatment responses were determined two hours later.

PROP (beta blocker) inhibits effects of chronic NTG administration

We showed previously that chronic intermittent administration of NTG produced acute and chronic hyperalgesia, which was prevented by the migraine preventive topiramate (15). To further validate chronic intermittent administration of NTG as a model of chronic migraine, we examined the effects of other migraine-preventive therapies. We first assessed the effects of chronic treatment with the beta blocker PROP. Mice treated with VEH-NTG showed a significant basal hypersensitivity (Figure 1(a), basal responses) that developed over time. They also showed an acute hyperalgesic response to each injection of NTG (Figure 1(b), post-treatment responses). Chronic treatment with PROP prevented the development of both NTG-induced basal hypersensitivity and post-treatment hyperalgesia (Figure 1).

VA (anticonvulsant) does not affect NTG-induced basal or acute hyperalgesia

We next examined the effects of the anticonvulsant VA. We tested two different doses of VA: 25 mg/kg, which is similar to a dose clinically, and 200 mg/kg, a dose previously shown to inhibit cortical spreading depression (CSD). Daily treatment with either dose of VA did not alleviate the basal hypersensitivity induced by chronic intermittent NTG administration (VEH-NTG vs. VA-NTG, Figure 2(a)). Chronic treatment with VA also did not affect acute hyperalgesia induced by NTG (Figure 2(b)). In addition, VA alone appeared to significantly reduce basal and post-treatment mechanical responses (VA-VEH vs. VEH-VEH).

AMIL (epithelial sodium channel (ENaC) inhibitor) inhibits effects of chronic NTG administration

After testing known migraine preventives, we next wanted to explore the effects of putative preventive compounds in our chronic model. The diuretic AMIL has recently been proposed as a novel migraine preventive (17). Daily treatment with AMIL (10 mg/kg) significantly attenuated NTG-induced basal hypersensitivity (VEH-NTG vs. AMIL-NTG, Figure 3(a)), and post-treatment responses (Figure 3(b)). Although AMIL did not result in a complete

inhibition of NTG-induced responses, as observed with PROP(Figure 1) or topiramate (15), it did inhibit basal and acute hyperalgesia by ~50%.

MEM (noncompetitive antagonist of N-methyl-D-aspartate (NMDA) receptors) mildly attenuates effects of chronic NTG administration

MEM, another novel potential migraine preventive, did not have a long-lasting effect on basal hypersensitivity or acute hyperalgesia induced by NTG. Daily administration of MEM (3 mg/kg) did not significantly alter the development of basal hypersensitivity by chronic NTG (VEH-NTG vs. MEM-NTG, Figure 4(a)). In terms of post-treatment responses, MEM initially blocked NTG-induced hyperalgesia on the first day, but this effect was not sustained over time (Figure 4(b)).

Chronic SUMA (5-HT1B and D agonist) models medication-overuse headache

To further characterize this chronic migraine model, we also examined the effect of chronic treatment with the acute migraine medication SUMA. As above, SUMA (0.6 mg/kg) was given once daily for 11 days, and mice were tested with NTG every other day starting on day 3. SUMA did not inhibit the development of basal hypersensitivity to NTG (VEH-NTG vs. SUMA-NTG, Figure 5(a)). Further, 11-day treatment with SUMA ultimately resulted in tolerance to the initial antihyperalgesic effects of this drug in post-treatment responses (Figure 5(b)). In addition, mice treated with SUMA alone also developed basal and post-treatment hypersensitivity similar to NTG treatment (VEH-NTG vs. SUMA-VEH, Figure 5(a) and (b)), consistent with worsening of the condition with regular use of the medication.

Discussion

A primary goal of this study was to further characterize a novel model of chronic migraine pain that uses chronic intermittent NTG. In a previous study, we had shown that the anticonvulsant topiramate effectively blocked both the development of basal hypersensitivity as well as NTG-evoked hyperalgesia (15). In this study we tested two known (PROP and VA) and two putative (AMIL and MEM) preventive compounds, all from distinct drug classes. The chronic NTG model indicated effectiveness of PROP (a beta blocker) and AMIL (an ENaC inhibitor), which inhibited both basal and evoked hyperalgesia. However, neither VA (an anticonvulsant) nor MEM (a non-competitive NMDA receptor antagonist) was effective at blocking NTG-induced hypersensitivity. Chronic daily administration of SUMA did not alleviate chronic migraine-associated hypersensitivity, and instead produced severe basal hypersensitivity, consistent with the phenomenon of medication-overuse headache.

The animal model that has been primarily used to screen preventive migraine treatments is the measurement of CSD events (14,18–20). CSD is the slowly propagating wave of depolarization followed by the suppression of neuronal activity, and it is considered to be the underlying mechanism for migraine aura (21). In rodents, multiple migraine-preventive medications with different pharmacological mechanisms have been shown to inhibit CSD (18), although not all medications that inhibit CSD are effective migraine-preventive therapies (19). In some of these studies, drugs were administered at high doses daily for four

to 17 weeks (18,19). Our results suggest that the chronic NTG model could be used as an additional tool for the relatively rapid screening of potential preventive compounds. Although the lack of an effect of VA in the assay indicates that it may not be 100% predictive of the efficacy of preventive therapies, the effects of established preventive medications with multiple mechanisms of action including topiramate and PROP indicate that the assay could be a meaningful component of a migraine-preventive therapy screening platform.

The beta blocker PROP effectively reduced both the development of basal hypersensitivity and acute NTG-evoked hyperalgesia. These results were similar to those observed with chronic treatment of topiramate (15). PROP is an effective migraine-preventive therapy (22,23), and chronic treatment with this drug is effective in the CSD model (18). Although it is not entirely clear how beta-adrenergic receptor blockers prevent migraine, one likely mechanism is by decreasing heightened noradrenergic signaling within brain regions regulating pain (24). Furthermore, in the periphery increased noradrenergic signaling at dural afferents and fibroblasts has also been shown to contribute to headache pain (25). Our results support the notion that NTG-induced chronic hypersensitivity is migraine specific, as beta-adrenergic antagonists treat migraine while *agonists* rather than antagonists appear to effectively block neuropathic pain states (26,27).

The novel migraine prophylactic AMIL inhibited both basal hypersensitivity and acute hyperalgesia within our chronic migraine model. AMIL is a diuretic known to block widely expressed ENaCs. This gene family includes the acid-sensing ion channels (ASICs), which are proton-gated channels that flux Na^+ and Ca^{2+} . The dose of AMIL used in this study was previously shown to decrease CSD, and neuronal responding in the trigeminal nucleus following dural stimulation (17). In addition, this compound also inhibited hyperalgesia induced by application of low pH solution to the dura (28). Furthermore, in a small open-label study, AMIL effectively reduced aura and medically refractory migraine (17). Our results support the multiple lines of evidence that indicate that AMIL could be an effective migraine-preventive treatment.

We did not observe an effect of VA or MEM within our NTG-based model of chronic migraine. The anticonvulsant VA is a clinically effective migraine-preventive therapy (24), and has been shown to prevent or acutely treat NTG-induced migraine in humans (29,30). The results with VA were somewhat surprising, as it shares many mechanisms of action with topiramate, which was effective in our model. We tested two doses of VA: a low dose that is comparable to that used in chronic migraine patients (25 mg/kg), and a high dose (200 mg/kg) to mirror a dose that was previously shown to inhibit CSD (18,19), although in these studies the drug was administered for at least four weeks. It is possible there may be differences in the effectiveness of this compound across species, as rats were used for the CSD experiments.

MEM is a noncompetitive antagonist at all subtypes of the NMDA receptor. In addition, it is also a noncompetitive antagonist at 5-HT₃, nAChR, and D₂ receptors. Acute administration of MEM was found to decrease CSD events, although higher doses of MEM were needed to see more robust effects (31). In addition, in a formalin model of trigeminovascular pain,

acute pretreatment with 10 mg/kg MEM decreased formalin-induced nociception and c-fos activation in the trigeminal nucleus (32). We tested a 3 mg/kg dose of MEM, as we observed Straub tail effects at higher doses suggesting activation of the opioid system. Initially, MEM blocked NTG-induced hyperalgesia; however, this effect was lost over time, which could reflect tolerance to chronic dosing. As with VA, it is possible that different treatment regimens or doses of MEM might prove to be effective in the chronic NTG model.

In this study we also tested chronic treatment with the acute migraine therapy SUMA. In two previous studies we had also tested this drug within the chronic NTG model, but SUMA was administered only following NTG administration (i.e. every second day over nine days, thus five drug days). We had found that acute SUMA alleviated NTG-evoked hyperalgesia (15,16), but did not rescue the basal hypersensitivity (15). In this study, SUMA was given daily for 11 days, and NTG testing occurred on days 3,5, 7, 9 and 11. Again SUMA did not block the induction of basal hypersensitivity by NTG. In addition, chronic daily SUMA alone produced basal hypersensitivity comparable to NTG injection. Medication-overuse headache is a major clinical problem (33), and has been observed with frequent use of SUMA (34). Administration of SUMA by minipump over multiple days has been shown previously to model medication-overuse headache (35,36). In this case chronic SUMA produced hyperalgesia in the head and peripheral regions concomitant with increased CGRP expression in trigeminal ganglia (35), and increased susceptibility to CSD (36). Consistent with these previous studies, our results indicate that daily intraperitoneal injection of SUMA can produce a medication-overuse phenotype.

Migraine chronification is likely due to adaptations in multiple regions of the central and peripheral nervous system. The CSD model measures changes in brain excitability that may underlie migraine and its progression, whereas the chronic NTG model quantifies behavior changes that likely reflect both peripheral and central mechanisms resulting in hypersensitivity. As with other models, the chronic NTG model is not perfectly predictive of efficacy of migraine-preventive therapies. Nonetheless, the effects of multiple migraine therapies with diverse pharmacology in this model indicate that it can be used to better understand basic mechanisms of migraine progression. Migraine headache is a heterogeneous condition, and although preventive medications are effective in some patients, they are not effective in all patients. Therefore, expanding the toolbox to incorporate multiple models with differing responsiveness to pharmacotherapies may help better elucidate the diverse mechanisms underlying headache across these diverse patient populations.

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Clinical implications

- Propranolol, a known migraine-preventive therapy, blocks acute and chronic hyperalgesia induced by chronic intermittent nitroglycerin (NTG).
- Amiloride, a potential migraine-preventive treatment, also inhibits acute and chronic NTG-induced hyperalgesia.
- Chronic sumatriptan produces hyperalgesia related to medication-overuse headache.
- The chronic NTG model can be used as an additional tool to screen novel migraine-preventive treatments.

Figure 1.

Chronic treatment with propranolol inhibited NTG-induced basal and post-treatment hyperalgesia. Male and female C57bl/6 J mice were treated with vehicle (VEH) or propranolol (PROP, 20 mg/kg, ip) daily for 11 days. (a) On days 3, 5, 7, 9, and 11, mice were injected with PROP or VEH, baseline responses were determined 15–20 min later, and mice were then injected with either VEH or NTG (10 mg/kg, ip). Two-way RM ANOVA, $p < 0.001$ drug and time, no significant interaction. (b) Post-treatment responses were assessed two hours following NTG administration. $p < 0.01$ drug and time, no significant interaction. $n = 7-9$ /group. The development of basal hypersensitivity and acute NTG-evoked hyperalgesia were blocked by treatment with propranolol. NTG: nitroglycerin; RM: repeated-measures; ANOVA: analysis of variance; ip: intraperitoneally.

Figure 2.

Chronic treatment with low or high doses of valproate (VA) did not inhibit basal or post-treatment NTG-induced hyperalgesia. Male and female C57bl/6 J mice were treated with vehicle (VEH) or valproate (VA) daily for 11 days. On days 3, 5, 7, 9, and 11, mice were injected with VA or VEH, baseline responses were determined 15–20 min later, and mice were then injected with either VEH or NTG (10 mg/kg, ip) and tested two hours later. (a) Basal responses for mice were treated with 25 mg/kg VA. Two-way RM ANOVA, $p < 0.001$ drug, time and interaction. (b) Post-treatment responses for mice treated with 25 mg/kg VA. Two-way RM ANOVA, $p < 0.001$ for drug, no significant effect of time or interaction. $n = 8$ /group. (c) Basal responses for mice treated with 200 mg/kg VA. Two-way RM ANOVA, $p < 0.01$ drug, time and interaction. (d) Post-treatment responses to 200 mg/kg VA. Two-way RM ANOVA. $p < 0.01$ drug, no significant effect of time or interaction. $n = 6$ /group, ** $p < 0.01$, *** $p < 0.001$. VA did not block migraine-associated pain in the chronic NTG model. NTG: nitroglycerin; RM: repeated-measures; ANOVA: analysis of variance; ip: intraperitoneally.

Figure 3.

Chronic treatment with amiloride (AMIL) attenuated NTG-induced basal and post-treatment hyperalgesia. Male and female C57bl/6 J mice were treated with vehicle (VEH) or AMIL (10 mg/kg, ip) daily for 11 days. (a) On days 3, 5, 7, 9, and 11, mice were injected with AMIL or VEH, baseline responses were determined 15–20 min later, and mice were then injected with either VEH or NTG (10 mg/kg, ip). Two-way RM ANOVA, $p < 0.01$ drug, time and interaction. (b) Post-treatment responses were assessed two hours following NTG administration. $p < 0.05$ drug and time, no significant interaction. $n = 12/\text{group}$, ** $p < 0.01$, *** $p < 0.001$. The development of basal hypersensitivity and acute NTG-evoked hyperalgesia were blocked by the novel migraine preventive AMIL. NTG: nitroglycerin; RM: repeated-measures; ANOVA: analysis of variance; ip: intraperitoneally.

Figure 4.

Chronic treatment with memantine (MEM) did not significantly alter basal or acute NTG-induced hyperalgesia. Male and female C57bl/6 J mice were treated with VEH or MEM (3 mg/kg, ip) daily for 11 days. (a) On days 3, 5, 7, 9, and 11, mice were injected with MEM or VEH, baseline responses were determined 15–20 min later, and mice were then injected with either VEH or NTG (10 mg/kg, ip). Two-way RM ANOVA, $p < 0.01$ drug and time, no significant interaction. (b) Post-treatment responses were assessed two hours following NTG administration. $p < 0.01$ drug, time and interaction. $n = 12/\text{group}$, $*p < 0.05$, $***p < 0.001$. Long-term, MEM did not block migraine-associated pain in the chronic NTG model. NTG: nitroglycerin; RM: repeated-measures; ANOVA: analysis of variance; ip: intraperitoneally.

Figure 5.

Chronic treatment with sumatriptan (SUMA) produced basal hypersensitivity and evoked hyperalgesia. Male and female C57bl/6 J mice were treated with vehicle (VEH) or SUMA (0.6 mg/kg, ip) daily for 11 days. (a) On days 3, 5, 7, 9, and 11, mice were injected with SUMA or VEH, baseline responses were determined 15–20 min later, and mice were then injected with either VEH or NTG (10 mg/kg, ip). Two-way RM ANOVA, $p < 0.01$ drug, time, and interaction. (b) Post-treatment responses were assessed two hours following NTG administration. $p < 0.01$ drug and time only. $n = 8/\text{group}$, *** $p < 0.001$. SUMA did not inhibit basal or acute NTG-induced pain, and chronic treatment with sumatriptan alone produced mechanical hypersensitivity. NTG: nitroglycerin; RM: repeated-measures; ANOVA: analysis of variance; ip: intraperitoneally.