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RESEARCH ARTICLE

Metabotropic glutamate receptor 5: a target for migraine therapy

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Abstract

Introduction: Many patients suffering from migraine gain little relief from existing treatments partly because many existing acute and preventive therapies used in migraine have been adopted from other neurologic conditions such as depression or epilepsy. Here, we present data supporting a new migraine-specific target, the $mGlu_5$ receptor. **Methods**: We studied the effect of $mGlu_5$ blockade using ADX10059, on neuronal firing in the trigeminocervical complex (TCC) and durovascular effects of nociceptive trigeminovascular activation in the anesthetized rat. The clinical potential of the $mGlu_5$ mechanism was tested with ADX10059 orally in a double-blind placebo-controlled, parallel group, clinical trial. **Results**: The negative allosteric $mGlu_5$ modulator ADX10059 attenuated dural vasodilator responses to meningeal stimulation in a dose-dependent manner, comparable to naratriptan, while the *N*-methyl-D-aspartate receptor blocker MK-801 had no effect. ADX10059 reduced responses of trigeminocervical neurons to dural stimulation, most strikingly affecting their spontaneous firing rate. Immunostaining identified $mGlu_5$ and not $mGlu_{1a}$ receptors in the TCC. The primary efficacy endpoint for the clinical trial, 2 h pain free, demonstrated a significant effect of ADX10059 375 mg, 17%, versus placebo, 5%. No serious adverse events were reported at the primary dose, with transient dizziness being the most common treatment-emergent event at 48%. **Interpretation**: Our findings provide preclinical and clinical proof of concept establishing $mGlu_5$ as a novel therapeutic target in the treatment of migraine. Although ADX10059 is unsuitable as a therapeutic candidate, because of hepatoxicity detected in a subsequent study, the data open a new direction for migraine research and therapy.

Introduction

Migraine is the most common neurological cause of disability worldwide.[1] It affects 12–18% of North Americans[2] and results in significant lost productivity with annual healthcare costs totaling billions of dollars.[3] Migraine treatment involves controlling acute attacks, with medicines such as triptans or serotonin $5\text{-}HT_{1B/1D}$ receptor agonists,[4] or with preventive agents, such as propranolol or topiramate.[5,6] Despite some advances, it is well accepted that more therapies are needed both in the acute treatment and preventive spaces. Moreover, the development of the calcitonin gene-

related peptide (CGRP)-based medicines demonstrates that a single molecular target can be exploited for acute and preventive treatment.[7,8] Thus, experimental models of nociceptive trigeminovascular activation[9–11] continue to serve an important role in new medicine discovery for both indications.

Metabotropic glutamate (mGlu) receptors are established modulators of inflammatory and neuropathic pain circuits in the spinal cord and brainstem.[12] Group I mGlu receptors, comprised of $mGlu_1$ and $mGlu_5$ subtypes, are Gq protein-coupled receptors linked to phospholipase C,[13] which in turn activates downstream extracellular signal-regulated kinase phosphorylation and reorganization of postsynaptic scaffolding proteins.[14] Although the role of mGlu receptors has been explored in peripheral and central pain models, few studies have examined their role in headache or migraine models.

Structures believed to be involved in migraine include the primary sensory fibers projecting from the trigeminal ganglia to the dural vasculature and to second order neurons located in the trigeminal nucleus caudalis and its cervical extension, the trigeminocervical complex (TCC). Excitatory transmission through the TCC is mediated predominantly by glutamate acting at ionotropic glutamate receptors.[15–17] Different glutamate antagonists targeting α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) ionotropic receptors have been tested in migraine patients with variable success and with considerable side effect issues.[18–20] Group I mGlu receptors are expressed in the trigeminal nuclei, periaqueductal gray, and thalamus, placing them in prime position to integrate incoming nociceptive and outgoing pain modulatory signals.[21,22]

Here, we examine whether modulation of $mGlu_5$ alters excitatory nociceptive transmission in the TCC or affects neurogenic changes in dural vasculature, as models that have previously been predictive of positive effects in migraine treatment.[11] We used ADX10059, which is a potent and selective negative allosteric modulator of the $mGlu_5$ receptor that does not have significant activity or binding affinity to other mGlu receptors, or other central nervous system (CNS) receptors, in particular, serotonin, dopamine, and γ-aminobutyric acid (GABA) receptors.[23] Translating this to a potential therapy, we report final data[24] on a randomized, placebo-controlled, parallel group study of ADX10059 in acute migraine to test whether $mGlu_5$ receptor modulation is an in-principle approach to migraine therapeutics.

Methods

Animal studies

All animal studies were approved by the Institutional Animal Care and Use Committee at UCSF and followed the NIH recommended guidelines for the care and treatment of laboratory animals. Animals were maintained at constant temperature and light cycles (12-h light/dark) and had free access to food and water at all times prior to the day of the experiment. Male Sprague Dawley rats (between 250 and 350 g) were anesthetized with inhaled isoflurane followed by intraperitoneal pentobarbital (Nembutal, 80 mg/kg) and maintained with intravenous (i.v.) propofol throughout all experiments. A femoral artery and both femoral veins were cannulated for blood pressure monitoring and i.v. administration of drugs, respectively. Animals were placed in a stereotaxic frame on a homeothermic blanket with body temperature monitored via a rectal thermometer and maintained between 36.5°C and 37.5°C. The trachea was cannulated and rats ventilated with oxygen-enriched air, 3–5 mL, 90 strokes per minute via a volume ventilator. End-tidal P_{CO_2} was continuously monitored and maintained between 3.5% and 5%.

Neurogenic dural vasodilation

A thinned parietal bone window was created over the dural meningeal artery until the vessel was clearly visualized.[25] Animals were allowed 1 h to recover while mineral oil was placed over the cranial window to prevent drying. Images of the artery were captured by microscope and projected via camera onto a television monitor. Changes in vessel diameter were measured by a video dimension analyzer and displayed via an online data acquisition system (CED Spike2 v5, Cambridge, UK). A bipolar stimulating electrode was positioned on the cranial window surface within 200 μm of the vessel of interest. A 10 sec train of 5 Hz stimulation was administered with 1 msec pulses between 10 and 40 V to achieve maximal vessel dilation. This maximal response voltage was used in the same animal throughout the experiment. After two stable control responses to dural electrical stimulation were obtained, i.v. vehicle control or drug treatment was dissolved in sterile water and slowly administered over 1 min. Electrical stimulation was repeated at 5–15 min intervals over 1 h after drug administration. Effects of electrical stimulation on dural vessel diameter were calculated as a percentage increase from prestimulation baseline diameters.

Extracellular recordings in the TCC

A craniotomy was performed over the parietal bone with exposure of the dura mater overlying the middle meningeal artery (MMA). Cervical spinal cord hemi-laminectomy was also performed, and the dura mater incised to expose the brainstem at the level of the caudal medulla. A tungsten recording electrode (0.5 MΩ, tip diameter 0.5 μm) was lowered into the brainstem with a piezoelectric motor

controller. Placement of the recording electrode into the V1 region of the trigeminal nucleus caudalis was guided by direct neuronal firing in response to cutaneous brush and pinch in the V1 ophthalmic dermatome. A bipolar stimulating electrode was placed on the dura mater adjacent to the MMA and square-wave stimuli (0.5 Hz) of 0.1–0.3 msec duration, 10–25 V were applied to activate trigeminal afferents. Extracellular recordings were made from neurons in the TCC activated by MMA stimulation or stimulation of cutaneous facial receptive fields. An average of three baselines for comparisons was used or compared to vehicle control recordings. The signal was amplified and passed through filters and a 60-Hz noise eliminator to a second-stage amplifier. This signal was fed to a gated amplitude discriminator and analog-to-digital converter and to a microprocessor-based computer for analysis. At the conclusion of experiments, the animals were given an overdose of Euthasol followed by bilateral thoracotomy.[26]

Drugs

ADX10059 (Addex Pharmaceuticals, Geneva, Switzerland) was administered and dissolved in sterile water at doses of 5 and 20 mg/kg. Naratriptan (Tocris, Bristol, UK) was administered at a dose of 10 mg/kg. MK-801 (Tocris, Bristol, UK) was administered at dose of 4 mg/kg.

Statistical analysis

SPSS (v22, IBM, Armonk, NY) was used for statistical analysis. To determine whether there was a significant effect across the 1-h time course pre- and postdrug administration, an analysis of variance (ANOVA) for repeated measures, with Bonferroni post hoc correction for multiple comparisons was performed, followed by Student's paired t test with significance at $P < 0.05$. If Mauchly's test of sphericity was violated, the Greenhouse-Geisser correction was used.

Immunohistochemistry

After deep anesthesia with isoflurane followed by Euthasol (Virbac, Fort Worth, TX, 1 mg/kg), rats were perfused transcardially with 100 mL of normal saline followed by 400 mL of 4% paraformaldehyde in phosphate-buffered saline (PBS). The caudal medulla and pons was removed as a single block and cut transversely into 50-μm sections using a vibratome. Cerebellar sections were obtained for immunostaining controls. Free-floating sections were incubated in blocking solution (PBS with 0.2% Bovine serum albumin (BSA), 5% normal goat serum, and 0.3% Tween20) for 2 h at room temperature, primary antibody (1:200, in PBS with 0.3% Tween20) was applied to sections for 48 h at 4°C. Sections were washed thoroughly in PBS with 0.3% Tween20 before overnight incubation at 4°C with secondary antibody (FITC or Cy5 anti-rabbit or anti-mouse antibodies [1:100], Jackson Immunolabs, Westgrove, PA) in PBS with 0.3% Tween20. Sections were rinsed and mounted on slides using VECTASHIELD® mounting medium (Vector Labs, Burlingame, CA). Images were visualized under a Zeiss Axioskop FS2 Plus (Pleasanton, CA) and images were taken with an Axiocam MRm running Neurolucida (MBF Biosciences, Williston, VT). Primary antibodies used were rabbit anti-$mGlu_5$ polyclonal antibody (Millipore, Billerica, MA, AB5675), mouse anti-$mGlu_{1\alpha}$ monoclonal antibody (BD Biosciences, San Jose, CA, 556331), and mouse anti-NeuN (AbCam, Cambridge, MA, ab104224). Double staining for $mGlu_5$ and NeuN was performed simultaneously.

All staining was performed with a no primary antibody condition to ensure minimal nonspecific staining by secondary antibody.

Clinical trial of ADX10059

The study was reviewed and approved by the respective Ethics Committee of each study center. The study was conducted according to the ethical principles of the Declaration of Helsinki.

Design

This was a multicenter, randomized, double-blind, parallel group, placebo-controlled Phase IIa study conducted between August and December 2006 in subjects affected by episodic migraine with and without aura (EUCTR 2006-001488-51-DE, Data S1). A total of 166 subjects were randomized (1:1) to receive either ADX10059 (375 mg) or placebo. The original planned dose was 500 mg ADX10059, but one of the first patients treated with this dose developed a serious adverse event (SAE). Consequently, the protocol was amended using the lower dose of ADX10059. Subjects were assigned to treatment arms by means of a block randomization design, stratified for each site. No single site was allowed to enroll more than 20% of the entire patient population. All clinical treatment staff were blinded to treatment assignment for the duration of the study. Code-break envelopes containing patient treatment allocation were provided to each site for emergency purposes only and the integrity of these envelopes was checked by the Study Monitor at routine monitoring visits.

Subjects

All subjects were screened for eligibility at their first clinical visit at one of 11 centers in Germany and two in the

United Kingdom. Eligibility was confirmed by telephone within 5 days of screening after which the patient was asked to treat their next moderate or severe migraine headache with study medication. Each patient took one dose of study medication for a single migraine attack. One migraine attack was to be treated within 8 weeks of enrollment. Patients who did not experience a migraine, or were not able or willing to treat a migraine attack, were withdrawn and considered not evaluable. Patients attended the clinic for a final follow-up visit where diary cards were reviewed and safety assessments made within 5 working days of treating their migraine headache. Data were collected in the diary cards using fixed time point (0, 0.5, 1, 1.5, 2, 3, 4, 24 h after taking study medication) and wristwatch data, documenting pain severity according to the IHS (International Headache Society) 4-point headache severity scale (0 = no pain at all, 1 = mild, 2 = moderate, and 3 = severe pain).

Study medication

The active treatment was formulated as size #0 opaque Swedish Orange hard gelatin capsules filled with ADX10059 citrate and placebo product was formulated as size #0 opaque Swedish Orange hard gelatin capsules filled with lactose. The study medication was taken orally with a glass of water and when possible study medication was taken on an empty stomach, that is, at least 2 h after food intake. The patients were instructed to abstain from eating and drinking for 2 h after taking the study medication. Patients were not to have taken analgesics, antiemetics or triptans, for 24 h prior to treating the migraine attack with study medication. If the patient did not have adequate relief 2 h after using the study medication, they could take approved rescue medications, which comprised simple analgesics, antiemetics or triptans.

Study population

Prior to beginning treatment, the aims, methods, and potential hazards of the study were explained to the patients verbally by the investigator, and in writing by means of a patient information sheet. Written informed consent was obtained from each individual participating in this study. The study included healthy male and female subjects aged 18–65 years with a diagnosis of migraine with or without aura.[27] Migraine history onset prior to age 50 years was required, and only patients who had between 2 and 8 moderate or severe migraine headaches per month were included. Subjects were excluded if they had more than 15 headache days per month; used sodium valproate or valproic acid within the previous 30 days; been administered any investigational drug up to 30 days before study entry; been taking medications metabolized exclusively by CYP1A2 and had a narrow therapeutic index; a known clinically significant allergy or hypersensitivity to ADX10059 or lactose; a history of significant medical or psychiatric condition that could have affected patient safety; and abnormal laboratory parameters at screening, in particular liver or renal function tests greater than twice the upper limit of normal. Patients using migraine prevention must have been on stable doses of the prophylactic agent for at least 12 weeks prior to study entry. Patients who were pregnant or breast feeding were excluded. Female patients who were of child-bearing potential were required to use adequate contraceptive methods.

Endpoints

The objectives of the trial were to investigate the efficacy, safety, and tolerability of the $mGlu_5$-negative allosteric modulator ADX10059 in the acute treatment of migraine. The primary endpoint for this study was the proportion of patients with initial moderate or severe pain who became pain free at 2 h post administration of study medication to treat the migraine attack. Secondary endpoints included evaluation of time to pain-free and/or pain relief, 24-h headache recurrence, presence of accompanying features of migraine, such as nausea, vomiting, photophobia, or phonophobia, use of rescue medication, patients' evaluation of study medication efficacy and adverse events.

Safety assessment

Adverse events were collected in the diary cards as well as by direct inquiry by the investigator. Vital signs, physical examination, and 12-lead ECG were collected at screening and follow-up, as were hematology, blood chemistry, and urine samples.

Statistical analysis

Determination of sample of size was based on an assumption of an active response of 33% and placebo response of 10%.[4] To reach 90% power, a sample size of 74 patients per group was needed. All primary and secondary parameters were analyzed using descriptive methods by applying chi-squared test or in case of small cell frequencies Fisher's exact test and Wilcoxon test. For all tests, a two-sided significance level of 5% was applied. The analysis of the primary endpoint was conducted with the Full-Analysis-Set[28] and was considered confirmatory, while the secondary analyses were considered exploratory.

Results

Animal studies

$MGlu_5$ blockade attenuates neurogenic dural vasodilation

The $mGlu_5$-negative allosteric modulator ADX10059 attenuates neurogenic dural vasodilation in response to meningeal stimulation in a dose-dependent manner. ADX10059 significantly reduced neurogenic vasodilation at a dose of 20 mg/kg by 19 ± 5% ($F_{5,25}$ = 2.68, P = 0.045, Fig. 1A), but not 5 mg/kg ($F_{5,25}$ = 0.54, P = 0.743, Fig. 1A). A reduction in vessel dilation was also achieved by naratriptan (29 ± 4%, $F_{5,25}$ = 4.19, P = 0.007, data not shown), an established migraine abortive. In contrast, inhibition of the ionotropic NMDA glutamate receptor by MK-801 does not alter vessel dilation in response to electrical dural stimulation ($F_{1.4,8.5}$ = 1.19, P = 0.331, Fig. 1B).

$MGlu_5$ blockade attenuates TCC neuronal activity

In support of the intravital microscopy data, systemic $mGlu_5$ inhibition reduces central nociceptive transmission through the trigeminal nucleus caudalis. In single unit neuronal recordings in the TCC of anesthetized rats, evoked activity from electrical stimulation of dura mater surrounding the MMA is significantly reduced by 24 ± 6% compared to the pretreatment baseline ($F_{2.1,16.8}$ = 4.63, P = 0.024, Fig. 2A–C). More strikingly, spontaneous activity of TCC neurons is significantly reduced by 49 ± 8% ($F_{1.89,15.0}$ = 6.94, P = 0.008, Fig. 2D). While overall activity in the TCC is blocked, sensory transmission in response to innocuous or noxious stimulation of the face, by brush or pinch in the V1 dermatome, is preserved (Fig. 2E).

$MGlu_5$ receptors are present in deep lamina of the TCC

Immunostaining using subtype-specific antibodies in rat brain slices reveals that $mGlu_5$, but not $mGlu_{1a}$ receptors predominate in deeper lamina of the trigeminal nucleus caudalis (TNC). Combined NeuN staining for neuronal cell bodies and proximal dendrites colocalizes with $mGlu_5$ in the TNC (Fig. 3C). In contrast, $mGlu_{1a}$ immunostaining is sparse compared to high levels of $mGlu_{1a}$ found in the cerebellum (Fig. 3D and F).

Clinical trial of ADX10059

Treatment groups in the clinical trial were well matched for demographic and baseline characteristics (Table 1) with a majority of female and Caucasian patients. The disposition of the subjects is contained in Figure 4.

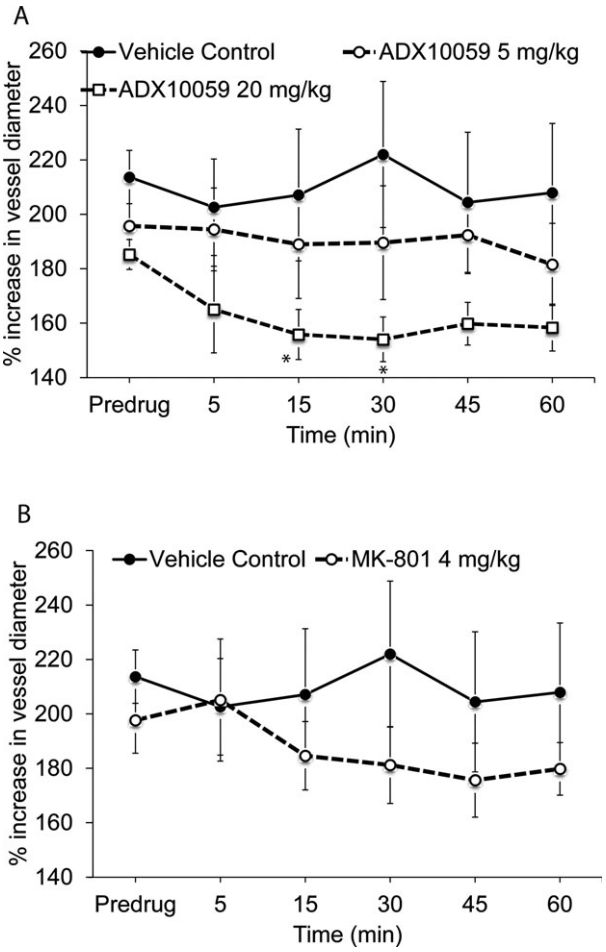

Figure 1. Intravital microscopy experiments demonstrating effects of glutamatergic agents on neurogenic dural vasodilation. Following control responses to electrical stimulation, rats were injected intravenously with the $mGlu_5$ inhibitor ADX10059 at 5 mg/kg (A, n = 6, clear circles), 20 mg/kg (A, n = 7, clear squares), sterile water (A and B, n = 7, filled circles), or MK-801 4 mg/kg (B, n = 7, clear circles) and electrical stimulation repeated after 5, 10, 15, 30, 45, and 60 min. *P < 0.025, significance compared to control response.

Primary endpoint

In the Full-Analysis-Set, a greater proportion of patients who treated their initial moderate to severe headache pain with ADX10059 375 mg (10/60, 17%) became pain free at 2 h compared to placebo (3/64, 5%). This trend was apparent at multiple time points after drug administration, and significant at 2 h using Fisher's exact test (P = 0.0397).

Secondary endpoints

In the Full-Analysis-Set, ADX10059-treated patients (6/60, 10%) were more likely to have a sustained pain-free response at 24 h than the placebo group (2/64, 3%;

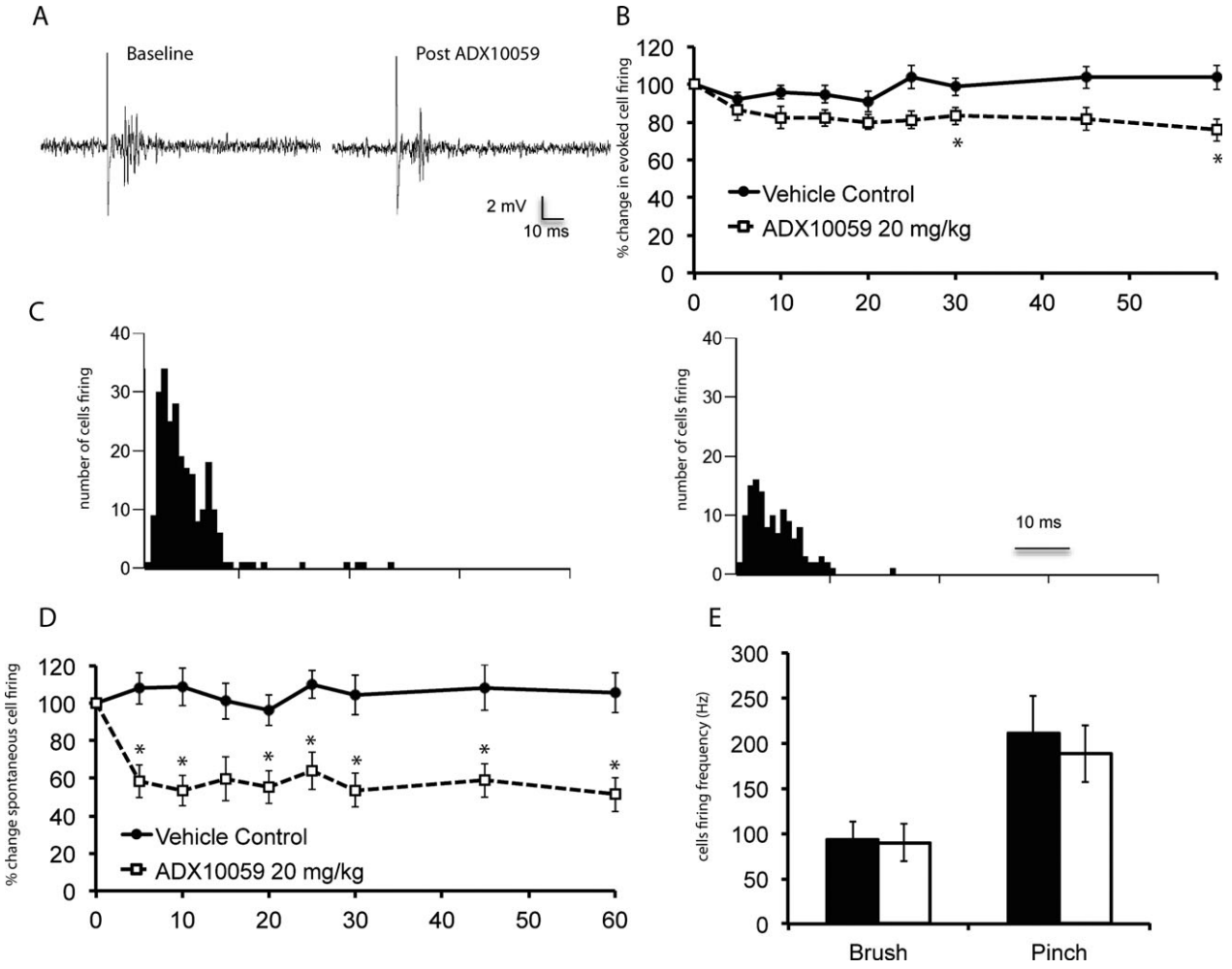

Figure 2. Single unit extracellular recordings in anesthetized rats. (A) Original tracing from a dural-evoked Aδ fiber neuronal response before and after ADX10059 (20 mg/kg). (B) Time course summary of stimulus evoked responses in the presence of vehicle control (sterile water or normal saline, $n = 9$) and ADX10059 (20 mg/kg, $n = 9$). (C) Sample poststimulus histogram (cumulative over 20 evoked responses) identifying Aδ fibers inhibited by ADX10059. (D) Time course summary of spontaneous cell firing in the presence of vehicle control or ADX10059. (E) Cell firing responses to light brush or noxious pinch over the V1 dermatome. Data are presented as mean ± SEM; $^*P < 0.0167$ in (B) and $^*P < 0.008$ in (D) when comparing to average of three baselines using Student's paired *t* test.

$P > 0.05$). There was no significant benefit of ADX10059 on functional impairment or associated symptoms of migraine, specifically nausea, photophobia, and phonophobia. In the ADX10059 group, 15 (25%) of 61 patients rated the medication fair, good, or excellent compared to 10 (15%) of 66 in the placebo group (NS). The proportion of patients taking rescue medication in the 24 h after dosing was 45/62 (73%) for ADX10059 and 48/66 (73%) for placebo.

Adverse events

There were no deaths or adverse event-related discontinuations during the study. Treatment-emergent adverse events (TEAEs) were reported more commonly in the ADX10059 group than in the placebo group. In the ADX10059 group, 79% (49/63) of patients experienced a total of 213 TEAEs compared to 36% (24/66) of patients who experienced 46 TEAEs in the placebo group (Table 2). One patient dosed with 500 mg experienced severe vertigo and was admitted to hospital and thus experienced a SAE. They recovered completely. The most commonly reported TEAE in the ADX10059 group was dizziness in 48% of patients. This was accompanied by nausea in 21% of patients. Also commonly reported in more than 10% of patients in the ADX10059 group were vertigo, vomiting, and blurred vision. Less than 10% of patients (10%) reported auditory or visual hallucinations

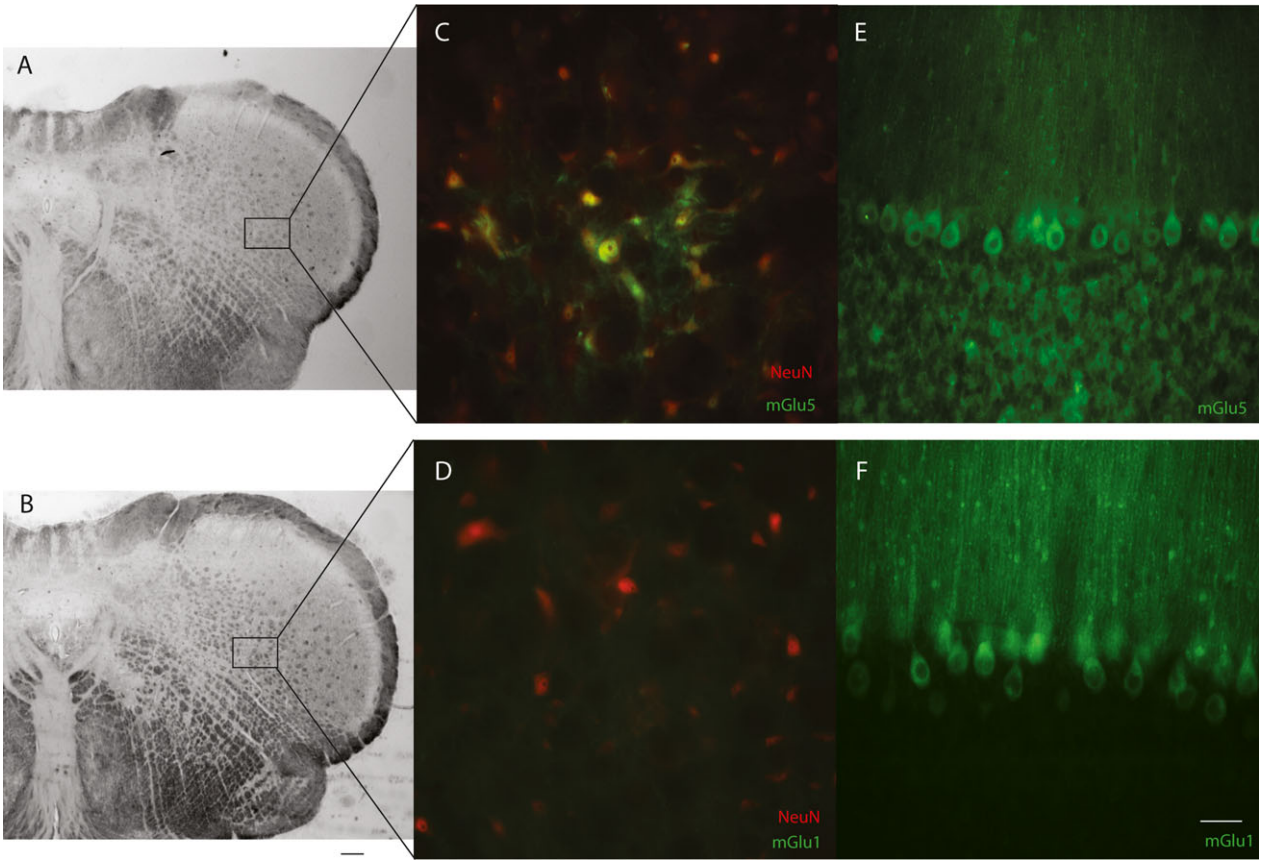

Figure 3. Immunohistofluorescent staining of group I mGlu receptors in naïve Sprague Dawley rats after intracardiac perfusion with 4% paraformaldehyde. (A, B) Light photomicrographs of medullary brain slices containing the TNC at 25× magnification. Immunostaining for (C) $mGlu_5$ (*green*) and (D) $mGlu_{1a}$ (*green*) colabeled with NeuN (*red*) at 200× magnification. Immunostaining for (E) $mGlu_5$ and (F) $mGlu_{1a}$ in the pyramidal layer of the cerebellum at 200× magnification. Scale bars = 200 μm (black), 50 μm (white).

Table 1. Subject demographics (safety cohort).

	ADX10059 *N* = 63 (±SD)	Placebo *N* = 66 (±SD)
Age (years)	41 ± 11	44 ± 10
Gender		
Male	12 (19%)	8 (12%)
Female	51 (81%)	58 (88%)
Race		
Caucasian	65	62
Non-Caucasian	1	1
Weight (kg)	71 ± 16	68 ± 11
Body mass index (kg/m^2)	24.9 ± 5.3	24.0 ± 3.5
Migraine days per month	3.6	3.7
Duration migraine history (years)	23 ± 12	25 ± 12
Migraine subtype		
With aura	12 (19%)	23 (35%)
Without aura	51 (81%)	43 (65%)

and diplopia. In the placebo group, TEAEs occurred occasionally and were reported with less than 5% when categorized by preferred term. ADX10059 administration did not lead to any clinically significant safety abnormalities, including vital signs, 12-lead ECG, hematological, or changes in biochemical laboratory values.

Discussion

Here, data are presented to demonstrate the presence and function of the mGlu receptor $mGlu_5$ in the TCC. Using two accepted models of dural nociceptive trigeminovascular activation,[11] it is shown that the negative allosteric modulator of the $mGlu_5$ receptor ADX10059 is active, suggesting a potential role in migraine. Furthermore, the clinical data from a randomized, placebo-controlled trial demonstrate efficacy of ADX10059 at the primary endpoint in the acute treatment of migraine. The role of mGlu receptors in nociceptive sensory transmission and central sensitization has been explored in various pain models. In particular, $mGlu_5$ has been shown to be involved in experimental inflammatory, neuropathic, and chronic hyperalgesia in animals.[29] Furthermore, activation

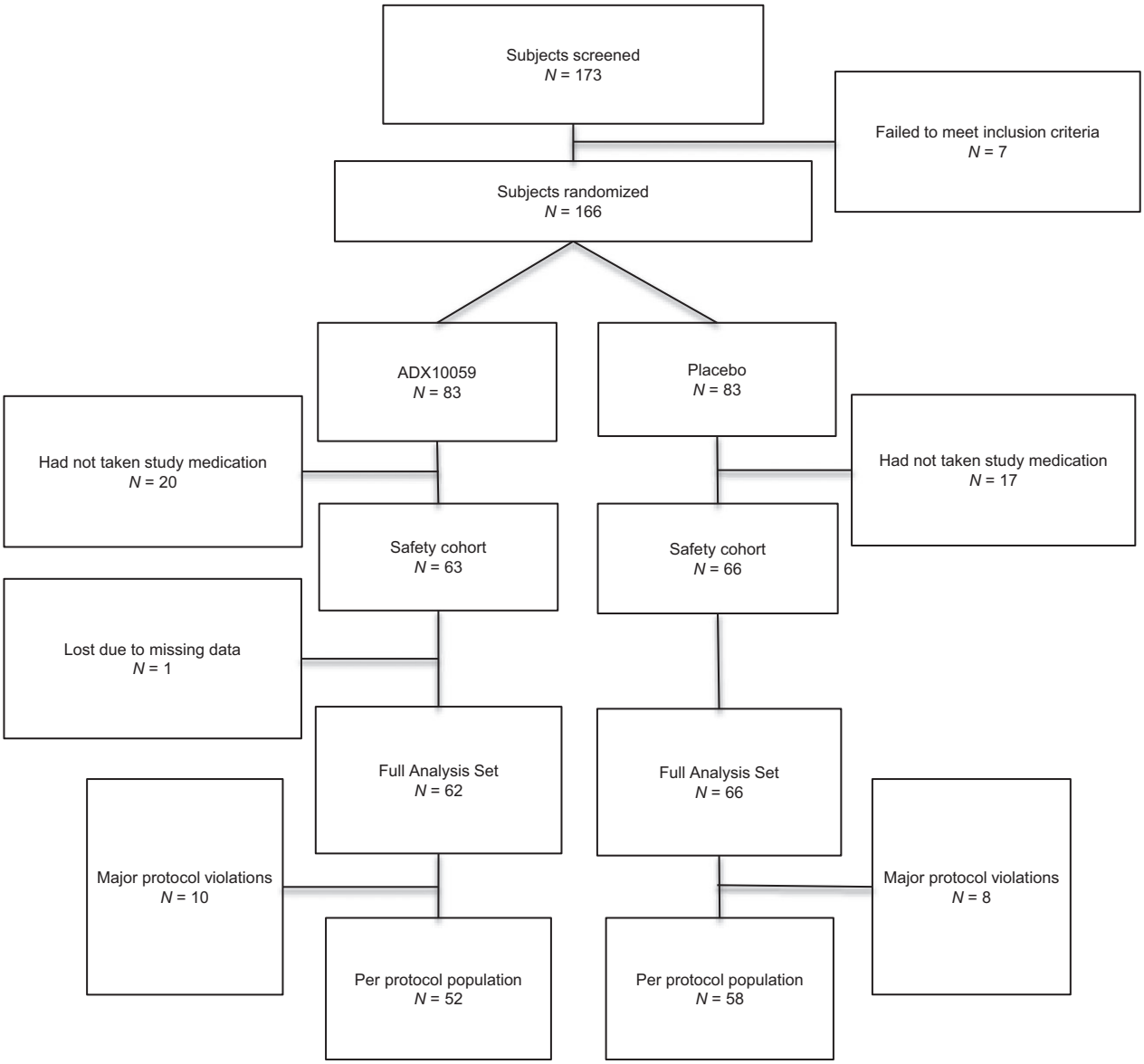

Figure 4. Patient flow in the study.

of group I mGlu receptors can promote central sensitization in dorsal horn neurons.[30] Our data, taken with our current understanding of the potential role of $mGlu_5$ receptors suggest that they are a new target for the treatment of migraine, perhaps best developed for prevention given its greater need.

This study establishes the role of $mGlu_5$ in trigeminal nociceptive processing using an animal model of dural trigeminovascular nociception relevant to migraine. Inhibition of $mGlu_5$ decreases nociceptive transmission and neurogenic vasodilation. The presence of $mGlu_5$ receptors in the TCC suggests a relevant site of action for ADX10059 in the caudal brainstem pertaining to trigeminal nociceptive processing, although it does not exclude actions at other sites. $MGlu_5$ receptors are also present in the trigeminal ganglion[31] and sensory afferent terminals in the periphery.[32] ADX10059 action at these sites may also contribute to the observed changes in dural vasculature and TCC neuronal activity. Interestingly, $mGlu_{1a}$ is not enriched in the TCC, suggesting a specific role for $mGlu_5$ in proximal pain modulation. However, $mGlu_1$ receptors are enriched in the thalamus[21] and may mediate higher order control of the pain experience.

ADX10059 demonstrates a statistically significant benefit over placebo in the proportion of pain-free patients at 2 h after dosing in this randomized, double-blind,

Table 2. Reported adverse events by body system (safety cohort).

	ADX10059 *N* = 63 (%)	Placebo *N* = 66 (%)
Subjects with adverse events	49 (78)	24 (36)
Total adverse events	213	46
Subjects with serious adverse events	1 (2)	0 (0)
System organ class		
Nervous system disorders	45 (71)	9 (14)
Gastrointestinal disorders	24 (38)	8 (12)
Psychiatric disorders	24 (38)	1 (2)
General disorders and administration site conditions	20 (32)	8 (12)
Eye disorders	16 (25)	3 (5)
Musculoskeletal and connective tissue disorders	5 (8)	2 (3)
Renal and urinary disorders	5 (8)	1 (2)
Cardiac disorders	4 (6)	1 (2)
Ear and labyrinth disorders	4 (6)	0 (0)

placebo-controlled, proof-of-concept study in migraine. The population of patients responding, 17%, is modest, although the placebo response was also small at 5% compared to a usual 10% for the pain-free endpoint in acute treatment migraine trials.[4] The 12% therapeutic gain difference between placebo and ADX10059 is the same as seen for the serotonin 5-HT_{1F} receptor agonist lasmiditan[33] and falls between the rates reported for the CGRP receptor antagonist telcagepant at therapeutic gains of 8% and 17% for the 150 and 300 mg doses, respectively.[34] The therapeutic gain for the 2 h pain-free endpoint for naratriptan 2.5 mg across its clinical development was 14%.[4] It should be recalled that the summary data are not "clinical" outcomes, since all patients responding are pain free, rather the outcome represents an integration of the pharmacokinetics of the drug and the population of migraine patients who may benefit from the therapy. Moreover, in migraine studies there are no published examples of false-positive studies using the pain free at 2 h endpoint, while there are a number of disproven approaches over the last decade, such as substance P receptor antagonists[35] or iNOS inhibitors.[36] Thus, the clinical data are broadly in line with other mechanisms that have gone on to be useful, or are currently in late stage development, and a false-positive result seems very unlikely. The study was not powered for the sustained pain-free or associated symptom endpoints since as a phase IIa study they were exploratory. Taken together, the development of this target offers a reasonable prospect as a novel therapeutic in migraine.

Because mGlu receptors act through second messenger signaling and not by direct ion channel modulation in comparison to AMPA, NMDA, and kainate receptors, mGlu receptors are thought to play a more modulatory role in sensory transmission. The effect of ADX10059 on spontaneous activity in the TCC may predict a role for $mGlu_5$ inhibition as a migraine prevention strategy. Moreover, the concept that medicines initially considered and developed for acute treatment cannot be used in prevention seems now substantially questioned, since CGRP mechanisms, both small molecule[7] and monoclonal antibodies,[37–41] have proven effective. Glutamate transmission modulation may be an attractive principle approach to migraine prevention if a suitable molecule can be developed.

One major drawback of ADX10059, and potentially the negative allosteric modulator approach, is the moderate prevalence of treatment-emergent adverse effects. Not surprisingly given the wide distribution of $mGlu_5$ throughout the nervous system, the majority of these adverse events were CNS related. Although there were no laboratory abnormalities after a single dose of ADX10059 in this study, subsequent clinical trials for longer term use of ADX10059 recognized significant hepatotoxicity,[23] with 6% (16/257) of patients in a migraine preventive study (NCT0082015) having alanine transaminase levels greater than five times normal, leading to termination of all studies with ADX10059. This adverse event likely is related to the structure of ADX10059, so the concept of $mGlu_5$ inhibition remains a valid mechanism for future drug discovery since other negative allosteric modulators at $mGlu_5$ have not had hepatic toxicity issues.[42]

In the past, other glutamate receptor antagonists have been used to treat migraine with variable success. LY293558 is a nonselective AMPA and kainate receptor antagonist that demonstrates antinociceptive effects in formalin-induced pain in animals[43] and capsaicin-induced hyperalgesia in humans.[44] LY293558 infusions were superior to placebo and comparable to subcutaneous sumatriptan in a randomized, double-blind, parallel group study in reducing moderate to severe pain during an acute migraine attack.[19] More recently, BGG492, an AMPA receptor antagonist, was assessed in a randomized, multicenter trial and improved headache and pain-free response compared to placebo in a subset of patients, but did not meet their proof-of-concept criterion of greater than 25% of responders at two time points.[18] The increased selectivity of LY293558 for kainate receptors over AMPA receptors compared to BGG492 may underlie clinical differences in efficacy of these two compounds. This suggests glutamatergic mechanisms in migraine vary by target receptor and offer a number of options to explore new therapies.

In summary, we have provided evidence of the $mGlu_5$ receptor in the trigeminocervical complex (TCC). Using two accepted models of dural nociceptive trigeminovascular activation, dural neurovascular stimulation and recording of neurons in the TCC activated by dural stim-

ulation, the data show the negative allosteric modulator of the $mGlu_5$ receptor ADX10059 is active. Furthermore, the clinical data from a randomized, placebo-controlled trial demonstrate efficacy of ADX10059 in the acute treatment of migraine comparable to some currently used therapies. No one pharmacotherapeutic mechanism in migraine has as yet controlled the needs for its acute treatment or preventive management in the estimated one billion patients worldwide. Our data establish negative allosteric modulation of the $mGlu_5$ receptor as a mechanism that could reduce in some manner the widespread and significant disability of this very common neurological disorder.

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Author Contributions

M. W. W., S. A., and P. J. G. conceived and designed preclinical studies. M. W. W. collected and analyzed experimental data, and wrote the first draft of the manuscript. S. A. and P. J. G. analyzed data and edited the manuscript. C. K. and M. W., when at Addex, and P. J. G. designed, analyzed, and have full access to the data from the clinical trial.

Conflict of Interest

M. W. W. reports nonfinancial support from Addex Pharmaceuticals S. A. has nothing to disclose. M. W. was an employee of Addex Pharmaceuticals at the time of the clinical trial and is no longer an employee. C. K. was an employee of Addex Pharmaceuticals at the time of the clinical trial and is no longer an employee. P. J. G. reports personal fees from Addex Pharmaceuticals, during the conduct of the study; grants and personal fees from Allergan, eNeura, Amgen; and personal fees from Autonomic Technologies, Inc., AlderBio, Pfizer, Dr. Reddy, Zosano, Colucid, Eli-Lilly, Avanir, Gore, Heptares, Nupathe, Teva, Cipla, Ajinomoto, Akita, Wells Fargo, Ethicon, Promius, MedicoLegal work in headache, Journal Watch, Up-to-Date, outside the submitted work; in addition, Dr. Goadsby has a patent "Magnetic stimulation for headache pending."

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Data S1. CONSORT checklist.

