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Original article

Utility of different outcome measures for the nitroglycerin model of migraine in mice

Sándor Farkas [a,b], Kata Bölcskei [a,c], Adrienn Markovics [a,c], Anita Varga [d], Ágnes Kis-Varga [d], Viktória Kormos [a], Balázs Gaszner [e], Csilla Horváth [a,d], Bernadett Tuka [f,g], János Tajti [f], Zsuzsanna Helyes [a,c,h,*]

[a] Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Szigeti u. 12, H-7624 Pécs, Hungary
[b] Research Division, Gedeon Richter Plc., H-1103 Budapest, Gyömrői út 19-21, Hungary
[c] János Szentágothai Research Centre, University of Pécs, Ifjúság út 20, H-7624 Pécs, Hungary
[d] Laboratory of Neuropharmacology, Pharmacological and Drug Safety Research, Gedeon Richter Plc., H-1103 Budapest, Gyömrői út 19-21, Hungary
[e] Department of Anatomy, Faculty of Medicine, University of Pécs, Szigeti u. 12, H-7624 Pécs, Hungary
[f] Neurology Department, University of Szeged, Faculty of Medicine, H-6725 Szeged, Semmelweis u. 6, Hungary
[g] MTA-SZTE Neuroscience Research Group, H-6725 Szeged, Semmelweis u. 6, Hungary
[h] MTA-PTE NAP B Chronic Pain Research Group, Faculty of Medicine, University of Pécs, Szigeti u. 12, H-7624 Pécs, Hungary

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ABSTRACT

Introduction: Majority of the work for establishing nitroglycerin (NTG)-induced migraine models in animals was done in rats, though recently some studies in mice were also reported. Different special formulations of NTG were investigated in various studies; however, NTG treated groups were often compared to simple saline treated control groups. The aim of the present studies was to critically assess the utility of a panel of potential outcome measures in mice by revisiting previous findings and investigating endpoints that have not been tested in mice yet.
Methods: We investigated two NTG formulations, Nitrolingual and Nitro Pohl, at an intraperitoneal dose of 10 mg/kg, in comparison with relevant vehicle controls, and evaluated the following outcome measures: light aversive behaviour, cranial blood perfusion by laser Doppler imaging, number of c-Fos- and neuronal nitrogen monoxide synthase (nNOS)-immunoreactive neurons in the trigeminal nucleus caudalis (TNC) and trigeminal ganglia, thermal hyperalgesia and tactile allodynia of the hind paw and orofacial pain hypersensitivity.
Results: We could not confirm previous reports of significant NTG-induced changes in light aversion and cranial blood perfusion of mice but we observed considerable effects elicited by the vehicle of Nitrolingual. In contrast, the vehicle of Nitro Pohl was apparently inert. Increased c-Fos expression in the TNC, thermal hyperalgesia, tactile allodynia and orofacial hypersensitivity were apparently good endpoints in mice that were increased by NTG-administration. The NTG-induced increase in c-Fos expression was prevented by topiramate but not by sumatriptan treatment. However, the NTG-induced orofacial hypersensitivity was dose dependently attenuated by sumatriptan.
Discussion: Our results pointed to utilisable NTG formulations and outcome measures for NTG-induced migraine models in mice. Pending further cross-validation with positive and negative control drugs in these mouse models and in the human NTG models of migraine, these tests might be valuable translational research tools for development of new anti-migraine drugs.

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1. Introduction

Migraine is a disabling headache disorder characterised by moderate to severe, intense throbbing or pulsating pain generally occurring on one side of the head and which may be aggravated by routine physical activity; other symptoms may include light or sound sensitivity, nausea and vomiting. Various forms of migraine affect approximately 18% of women and 6% of men (Estemalik & Tepper, 2013). Although acute treatment of migraine is well manageable by triptans, there are many patients who cannot tolerate triptans or would need preventive medication. However, efficacy and tolerability of the preventive armamentarium, including antiepileptics, such as topiramate and valproate, and

Abbreviations: 5-HT, serotonin; CGRP, calcitonin gene related peptide; NTG, nitroglycerin; NO, nitrogen monoxide; nNOS, neuronal nitrogen monoxide synthase; TNC, trigeminal nucleus caudalis; TRP, transient receptor potential; TRG, trigeminal ganglion; PBS, phosphate-buffered saline; PBS-T, Triton-X containing PBS; DAB, diaminobenzidine; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous.

* Corresponding author at: Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Szigeti u. 12, H-7624 Pécs, Hungary.
E-mail addresses: farkass1@gmail.com (S. Farkas), kata.bolcskei@aok.pte.hu (K. Bölcskei), adri.markovics@gmail.com (A. Markovics), anivarga@richter.hu (A. Varga), i.kisvarga@richter.hu (Á. Kis-Varga), viktoria.kormos@gmail.com (V. Kormos), balazs.b.gaszner@aok.pte.hu (B. Gaszner), horvathcsiti@gmail.com (C. Horváth), tukabernadett@googlemail.com (B. Tuka), tajti.janos@med.u-szeged.hu (J. Tajti), zsuzsanna.helyes@aok.pte.hu (Z. Helyes).

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beta-adrenergic receptor blockers are unsatisfactory (Silberstein et al., 2012). Therefore, a great unmet need exists for novel effective, safe and well-tolerated pharmacotherapies of migraine.

Although recent research has revealed numerous details of the mechanisms participating in migraine generation, the primary initiating phenomena, the underlying neural and vascular mechanisms and their interrelationships are not understood and are surrounded by serious debates on various proposed theories, such as the vascular, the neural and the neurogenic inflammation theories of migraine (Ashina, 2012; Messlinger, Fischer, & Lennerz, 2011; Moskowitz, 1993; Noseda & Burstein, 2013).

In line with the lack of a well-established theoretical background, numerous models have been proposed as useful tools for testing novel antimigraine drug candidates in animals or in human pharmacodynamic studies. Out of these, the most widely studied and accepted one is the nitroglycerin (NTG)-induced model of migraine (Olesen & Jansen-Olesen, 2012). NTG administration causes an immediate headache, stronger in migraine sufferers than in healthy subjects, and a delayed migraine-like headache only in migraineurs (Ashina, Hansen, & Olesen, 2013; Olesen, 2008; Thomsen, Kruuse, Iversen, & Olesen, 1994). The immediate headache in healthy volunteers was significantly attenuated by sumatriptan (Iversen & Olesen, 1996) and the delayed headache incidence in migraineurs was reduced by valproate (Tvedskov, Thomsen, Iversen, et al., 2004).

The majority of the work for establishing NTG-induced migraine models in animals was done in rats detecting the effect by various outcome measures, such as increased cerebral- and more controversially meningeal blood flow or blood vessel diameters (Gozalov, Jansen-Olesen, Klaerke, & Olesen, 2008; Greco et al., 2011; Pryazhnikov et al., 2014; Read, Manning, McNeil, Hunter, & Parsons, 1999; Srikiatkhachorn, Suwattanasophon, Ruangpattanatawee, & Phansuwan-Pujito, 2002), increase in c-Fos protein expression in the trigeminal nucleus caudalis (TNC) (Knyihar-Csillik et al., 2008; Pardutz, Krizbai, Multon, Vecsei, & Schoenen, 2000; Ramachandran et al., 2012; Tassorelli & Joseph, 1995), increase in neuronal nitric oxide synthase (nNOS) protein expression in trigeminal ganglia (TRGs) and/or TNC (Dieterle, Fischer, Link, Neuhuber, & Messlinger, 2011; Pardutz et al., 2000; Srikiatkhachorn et al., 2002), electrophysiological detection of increase in neuronal activity of TNC neurons (Jones et al., 2001; Koulchitsky, Fischer, & Messlinger, 2009) and hypersensitivity to pain either on the paw/tail or in the facial (trigeminal) region measured by behavioural responses to chemical, mechanical or thermal stimuli (Di et al., 2015; Greco et al., 2015; Tassorelli et al., 2003; Tassorelli, Greco, Wang, Sandrini, & Nappi, 2006). Whereas the use of rats has several advantages, there are also reasonable considerations in favour of utilising mice, such as better availability of transgenic animals or the need to use much lower amounts of expensive substances for in vivo studies. However, much more limited experience exists concerning the NTG model in mice. Recently reported data indicated that increased c-Fos expression (Bates et al., 2010; Goloncser & Sperlagh, 2014; Markovics et al., 2012), thermal hyperalgesia (Bates et al., 2010; Goloncser & Sperlagh, 2014) and mechanical allodynia of the paw (Bates et al., 2010; Pradhan et al., 2014) are utilisable outcome measures of NTG-induced changes in mice. In addition, our previous study suggested that increased cranial blood flow and light aversive behaviour are also suitable endpoints to detect NTG-induced changes in mice (Markovics et al., 2012). The aim of the present studies was to critically assess a panel of utilisable outcome measures in mice by revisiting previous findings, as well as by adding endpoints that have not been tested in mice yet, e.g. nNOS expression in the TRG and TNC, as well as pain hypersensitivity of the face, which formally might be a more relevant indicator of migraine than paw hyperalgesia.

There was another confounding factor in previous studies, which determined our goals. In various studies different formulations of NTG were used, which were composed either for infusing or for sublingual spray application in patients, and the exact compositions of these often remained elusive. Several different NTG formulations contain propylene glycol and ethanol or propylene glycol and glucose (e.g. Nitrocine — https://www.medicines.org.uk/emc/medicine/1889); others (e.g. Nitrolingual) have even more complex vehicle without description of the exact composition. Some relatively dilute aqueous solutions of NTG (Nitro Pohl and Nitronal) contain only 5% glucose and mild acidification. Some of the abovementioned constituents (e.g. ethanol) may be assumed not to be entirely inert in studies of central nervous system functions. Nevertheless, many previous studies compared the effects of formulated NTG to a saline group instead of using an appropriate vehicle control (Bates et al., 2010; Di et al., 2015; Srikiatkhachorn et al., 2002; Tassorelli et al., 2003, 2006) and in lack of control vehicle we did the same in a previous study (Markovics et al., 2012) using Nitrolingual formulation of NTG. However, in the present studies, to establish well-controlled NTG models, we intended to use appropriate vehicle controls. For this purpose we clarified the composition of Nitrolingual and composed an appropriate vehicle for control experiments. In addition, we started the studies with investigating two different formulations, Nitrolingual and Nitro Pohl, which latter we considered as the possibly most inert one from the assortment.

2. Methods

2.1. Animals

Male CD1 mice (N = 48) weighing 22–28 g were used for cranial blood flow and immunohistochemistry studies to preserve comparability to a similar previous study (Markovics et al., 2012). They were bred in the Laboratory Animal House of the Department of Pharmacology and Pharmacotherapy of the University of Pécs. For the behavioural experiments, male NMRI mice (N = 142) weighing 25–35 g were used. In our experience this strain is more tranquil and suitable for behavioural and particularly for reliable pain threshold studies than the CD1 strain. NMRI mice were obtained from TOXI-COOP Zrt (Budapest, Hungary). The animals were housed under standardised conditions (22 ± 2 °C room temperature) with an artificial 12/12 h light/dark cycle (lights on 06.00–18.00 h, humidity 55 ± 15%). Food (ssniff® R/M-H autoclavable; ssniff Spezialdiäten GmbH, Germany) and tap water were available ad libitum. In all behavioural experiments, the animals were acclimatised to the laboratory for at least 30–60 min before testing and were used only once. Experiments were performed in accordance with EU Directive 2010/63/EU for animal experiments. All the procedures involving animals were reviewed and approved either by the Richter Institutional Animal Care and Use Committee or the Ethics Committee on Animal Research of the University of Pécs. The number of animals and intensities of noxious stimuli used were kept to the minimum necessary to demonstrate consistent effects of the drug treatments.

2.2. Drug treatments and control vehicles

Two NTG preparations were used: Nitrolingual aerosol and Nitro Pohl solution for infusion (both obtained from Pohl-Boshkamp GmbH, Germany). Nitrolingual aerosol was freshly sprayed out into a foil-coated bottle on each experimental day. The solution contains 7.7 mg/ml NTG and was administered at a dosing volume of 1.3 ml/kg for a dose of 10 mg/kg intraperitoneally (i.p.). The vehicle control solution for Nitrolingual was clarified by data mining in patent literature (US 2011/0240508) and compounded at Gedeon Richter Plc. and comprised (in % *w*/*w*): Miglyol 812 (caprylic/capric triglyceride, Sasol Germany GmbH) 77.3%, Ethanol (96% Ph Eur.) 20%, Imwitor 988 (glyceryl caprylate, Sasol Germany GmbH) 2% and Peppermint oil (Sigma-Aldrich) 0.7%.

One ampoule Nitro Pohl solution for infusion was freshly opened on each experimental day. The solution contains 1 mg/ml NTG and was administered at a dosing volume of 10 ml/kg for a dose of 10 mg/kg i.p. In addition to NTG, the aqueous Nitro Pohl solution contains 49 mg/ml

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