Experimental models of migraine both in vitro and in vivo

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Abstract

Animal models have been critical to our understanding of the neurobiology of migraine. Model systems have the advantage of being able to control experimental variables to a much greater degree than in human studies. Over the past decade, a number of animal models of migraine have been developed. However, each animal model of migraine has its own merits and demerits. It is imperative that animal model selection shall be based on the neurobiological mechanisms of migraine one chooses to study. The review summarizes numerous animal models of migraine. In our review, we classified migraine models into five categories: i) models based on anastomoses and isolated blood vessels; ii) models based on neurovascular involvement; iii) Superior Sagittal Sinus stimulation; iv) nitroglycerin induced model; v) genetically modified mouse models. This review discussed above mentioned models covering implications of each model.

Introduction

Migraine is a common neurological disorder which causes significant personal and societal burdens due to high prevalence, loss of productivity and the cost of treatments which are not always effective for patients.1 Furthermore, there is relatively little basic research conducted on migraine2 and as a result available treatment options and understandings of this condition are limited. Migraine is diagnosed in women three times more frequently than men1 and while the mechanisms behind these sex differences is not well understood. Migraine is the most common primary headache syndrome during childhood and adolescence. However the pathophysiology of migraine is not fully understood. The proposed theory for classical migraine pathophysiology is trigeminovascular theory. The propagation of the cortical spreading depression to the pain sensitive trigeminal sensory fibres is believed to induce the headache. Migraine is a multi-factorial primary headache disorder, is characterized by unilateral intense and pulsatile headaches.4

Models based on anastomoses and isolated blood vessels

Based on anastomoses

In the late 1930’s, Harold Wolff became the first researcher to place migraine on a scientific basis, Wolf measured the diameter of the extracranial (temporal) arteries in patients suffering migraine attacks and found them to be dilated. These patients were treated with vasoconstrictors (ergotamine) which relieved the pain and decreased the arterial dilation.5 Thus dilation of cranial blood vessels including carotid arteriovenous anastomoses is involved in headache phase of migraine. Arteriovenous anastomoses are precapillary communications between the arteries and veins; they are predominantly located in the head skin, ears, nasal mucosa, eyes and dura mater in several species, including humans and pigs.6 Indeed, migraine patients also experience facial paleness, a decline in facial temperature, and rise in temporal artery pulsations and swelling of the frontal vein on the side of the headache besides their headaches.7,8 Therefore, Heyck determined the oxygen saturation difference between arterial and external jugular venous blood samples (A-V SO2 difference) during and after the headache phase of migraine and compared it with those of healthy controls. The A-V SO2 difference was decreased during the headache phase of migraine, possibly due to dilatation of carotid arteriovenous anastomoses, which was normalized after spontaneous or drug induced mitigation of the headache.9

On account of this hypothesis, in vivo animal models of pharmacologically evoked carotid vasodilation have been developed.10,11 For instance, in conscious pigs, shunting of blood by carotid anastomoses accounts for less than 3% in the jugular venous circulation whereas this fraction is augmented up to 80% under pentobarbital anesthesia as detected by radioactive microspheres.12 As a result, opening of the carotid arteriovenous anastomoses during migraine shunts a large quantity of oxygenated blood directly into the veins thus resulting in facial pallor, reduction of skin temperature and raise in vascular pulsations. The above mentioned shunt model has been functional effectively to evaluate the blood flow effects of abortive anti-migraine drugs. Those drugs that demonstrate desired effects on carotid A-V anastomotic blood flow in pig or on the external carotid bed in dogs (e.g. triptans) show abortive activity in acute migraine.10,11

The major advantage of this model is that different vascular beds can be studied simultaneously in order to evaluate the cranioselectivity of anti-migraine drugs.10 The major limitation is that this model will only pick up potential anti-migraine drugs acting via vascular mechanisms.13 Another limitation is that this model cannot reflect the complexity that constitutes migraine disorder. However, it remains to be determined in which ways, if any, A-V shunting relates to migraine pathogenesis or in which ways shunt opening and closing evokes pain. It appears that 5-hydroxytryptamine (5-HT) 1B/D receptors, relevant to the triptan effect, cause constriction within a relevant population of blood vessels opening and closing A-V shunts. Therein lies the value of this model and its impact on migraine till date.14

Based on isolated blood vessels

In vitro studies on isolated blood vessels,
using vascular segments mounted in organ baths have proved to be of high value, particularly in characterizing the pharmacologic profile of prospective anti-migraine drugs such as triptans or ergot derivatives. Any blood vessel with a minimal internal diameter of about 75 microns can be studied. However, the following specific blood vessels have been frequently used in migraine related studies are bovine cerebral arteries and dog or rabbit saphenous vein as these preparations possess certain similarities with human cranial or coronary arteries. Studies on isolated blood vessels offer several advantages: i) drug receptor interactions at equilibrium, ii) the possibility to carry out a detailed pharmacological analysis, mounting multiple segments of blood vessel in parallel, iii) no influence by pharmacokinetic factors, iv) exclusion of central and autonomic mechanisms as well as the effects produced by circulating hormones, distending pressure, etc., v) the possibility to remove the endothelium from the blood vessel, which provides information whether the receptors are present on the endothelium or in the vascular smooth muscle of arteries, vi) the possibility to gain insight into downstream signaling, such as mechanisms of second messengers and most notably vii) the possibility to study human preparations. In vitro models using isolated blood vessels have been applied successfully to test the efficacy of anti-migraine drugs on constriction of cranial arteries. The therapeutic efficacy of acutely acting anti-migraine drugs is most likely, at least for a major proportion, mediated by constriction of dilated cranial arteries. Cumulative concentration response curves are used to determine the vasoconstrictor potency and efficacy of the prospective anti-migraine agent although in vitro vascular models do not reflect the complexity of migraine nor inform us about pharmacokinetics, they became the workhorse for the development of triptan drugs and continue to provide detailed information about vascular smooth muscle pharmacology. However, it still remains unclear whether the receptor most relevant to the abortive migraine effect resides on vascular smooth muscle. Human isolated coronary arteries are useful in analyzing the coronary side effect potential of anti-migraine drugs. Although the chest symptoms (chest pressure, tightness and pain) commonly experienced after the use of anti-migraine drugs are in most cases not likely to be due to coronary vasoconstriction, cardiac ischemia after anti-migraine drugs has indeed been reported.

Models based on neurovascular involvement

Neurovascular models focus on the interplay between neurovascular changes observed during migraine attacks and the consequences of trigeminovascular activation. A basic assumption is that migraine pain is caused by activation of trigeminal axons near blood vessels and surrounding meningeal tissues. Several techniques have been developed to study the effects of activation of the trigeminovascular system. By doing so, neuroinflammatory peptides are released antidromically from perivascular nerve fibres (calcitonin gene related peptide – CGRP – SP and NK-A) to promote vasodilatation,24 activation of the endothelium with increased transendothelial transport, mast cell activation, enhanced local platelet aggregation and adhesion.25,26 All these mechanisms promote extravasation of plasma protein into dura mater, although, not all techniques reproduce this phenomenon. At the same time, the orthodromic activation of the C-fibers releases glutamate, substance P, CGRP, NKA in the Sp5e with activation of second order neuron.

Trigeminal ganglion stimulation

CGRP from perivascular axons can be blocked by Triptans, ergot alkaloids, indomethacin, and also acetylsalicylic acid. Plasma protein leakage is detected quantifying the ratio of radio isotopes [I-BSA] or dyes (Evans Blue) in the perfused meninges of the stimulated versus non-stimulated side. Blood flow measurements using laser Doppler recordings are another endpoint used to indirectly assess neurovascular responses following electrical stimulation of the dura mater. Building upon this model and the concept of neurovascular dilation, intravital microscopy was developed to analyze diameter changes in dural vessels and for studying the consequences of activating the peripheral branches of the trigeminovascular system in this model, electrical field stimulation causes a reproducible dilation of dural and pial blood vessels via release of CGRP from pre-synaptic trigeminal nerve endings. Inhibition of neurogenic dural vasodilation can assess anti-migraine drug efficacy, as has been shown for the Triptans and a CGRP receptor antagonist.

Meningeal stimulation

Meningeal stimulation by autologous blood or capsicain activates primary sensory nerve fibers and induces c-fos expression in a dose-dependent manner. Stimulation of sensory nerve fibres results in the release of neuropeptides in both the central and peripheral nervous system. Studies show that sensory nerves innervating the cerebral vasculature contain substance P and CGRP. Strassman and colleagues demonstrated that topical meningeal application of an inflammatory soup (histamine, 5-HT, prostaglandin E2 at pH 5.0 and bradykinin) sensitizes meningeal afferents, activating Sp5C neurons to mechanical stimuli. Early administration of sumatriptan effectively blocks the enhanced sensitivity to mechanical stimuli. In this model, a small catheter is placed into the cisterna magna through the atlanto-occipital membrane of anesthetized rats, mice or guinea pigs. A test compound or vehicle is administered intravenously or intraperitoneally and approximately 1h after catheter placement an irritant substance, either autologous blood; carrageenin or capsicain is injected intracranially. End points used in this model is Plasma protein extravasation and expression of c-Fos mRNA and Fos immunoreactivity within the superficial laminae of trigeminal nucleus caudalis.
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**Superior sagittal sinus stimulation**

Another major model is superior sagittal sinus stimulation in cats, monkeys and rats. The superior sagittal sinus stimulation (SSS), the tributary veins and adjacent dura are intracranial structures densely innervated by a separate branch of the ophthalmic division of the trigeminal nerve, the tentorial nerve. Stimulation of the SSS electrically or mechanically induces the release of neuro peptides in a pattern similar to that observed during migraine attacks. This model gives valuable information of the site of action of anti-migraine drugs in a non-inflammatory paradigm, for example sumatriptan does not inhibit c-fos induced by SSS stimulation, while zolmitriptan does. This argues in support of a central mechanism of action of zolmitriptan, which is lipophilic, without disposal of peripheral mechanisms. The fact that sumatriptan was only effective in this model after BBB disruption strongly supports a peripheral mode of action. This model is preferred by those who do not believe that neurogenic inflammation underlies migraine. C-fos gene is expressed in this type of models has greatly improved the understanding of pathophysiology and pharmacology of trigemino vascular system establishing itself as one of the most effective experimental model.

**Nitroglycerin induced model**

By far, the best validated and most studied human migraine model uses intravenous infusion of nitroglycerin, also called glyceryl trinitrate (GTN). It is now generally accepted that infusion of GTN induces migraine attacks in migraineurs that are indistinguishable from spontaneous attacks in normal volunteers without migraine. GTN does not dissolve in saline and usually must be dissolved in a mixture of alcohol and propylene glycol. Many studies using intraperitoneal GTN have, however, used saline for control and not vehicle. Because very large amounts of GTN are necessary, the amount of injected alcohol and propylene glycol are considerable. Furthermore, it is unknown what the effect of alcohol and propylene glycol into the peritoneum might be. Hence, specificity of the responses obtained in most of these animal experiments is questionable. The infusion of nitroglycerin (NTG) induces an inflammatory state in perivascular meningeval tissues of rat via the activation, inter alia, of nuclear factor kappa B (NF-kB). The huge doses of GTN induce a marked and prolonged blood pressure decrease in rats. Although it has been reported that intraperitoneal injection of 10 mg/kg GTN has no effect on blood pressure in mice many attempts have been made to develop animal models predictive of efficacy of anti-migraine drugs. We are not there yet but development of such a model seems to be one of the most important avenues of headache research today.

**Genetically modified mouse models**

Previously, it has been shown that genetic factors play an important role in migraine pathophysiology, the first two migraine genes, CACNA1A and ATP1A2 were discovered in families with hemiplegic migraine a severe subtype of migraine with aura. CACNA1A encodes alpha 1 subunit of voltage gated calcium channel; the second migraine gene ATP1A2 encodes alpha 2 subunit of sodium potassium pump.

**Transgenic knock in mouse models**

Two knock-in mouse models of FHM have been generated to study the functional outcomes of FHM mutations and to identify pathophysiological mechanisms potentially involved in FHM and hence possibly also in non-hemiplegic migraine.

By use of a gene-targeting approach, the human pathogenic Arg192Gln or Ser218Leu missense mutation were inserted into the mouse orthologous Cacna1a by homologous recombination. Arg192Gln was chosen because it is associated with a mild pure FHM phenotype without additional clinical features, modeling migraine as closely as possible. Ser218Leu was selected because it is associated with probably the most severe FHM1 phenotype. Comparison of functional changes in both mouse models enabled a disease severity dependent analysis and the differentiation of possible pathways for migraine and its associated features. Identification of responsible mutations in patients suffering from FHM through a gene targeting approach supports the concept that FHM, and possibly a few other more common types of migraine, are ionopathies that lead to cortical network hyperexcitability in patients with migraine.

**Conclusions**

Current animal models of migraine test only part of the whole rather than entire picture. Each model focuses on a particular pathophysiological system. Hence, one needs to test the drug candidate in various animal model systems. If a molecule was found to be ineffective in a single model, it does not mean that drug is ineffective. It may be possible that drug is not working in a mechanism of animal models that one has chosen. Each model has its unique features. Comparisons, interpretations of results of various animal models would help researcher to draw meaningful conclusions.

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