Animal Models of Migraine

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1 Introduction

Migraine is defined as a multifactorial and episodic disorder which features unilateral, hemicranial, throbbing headache often accompanied by nausea and vomiting and aggravated by movement, sound, and light. Migraine has two major clinical subtypes: migraine without and with aura. One-third of patients with migraine experience aura, that is, transient focal neurological symptoms like sensory or motor deficits. Migraine is a chronic and disabling disorder of the brain that affects up to 15% of the population worldwide. It has a devastating consequence on the quality of a patient life and is estimated to cost $19.6 billion and €27 billion in the USA and Europe per year, respectively.

The clinical depiction of a migraine attack suggests that a series of processes including vascular, neuronal, and biochemical elements occur at different sites. A significant contribution has been made in understanding the pathophysiology of this condition, but the exact pathophysiology of migraine is still not fully understood. It is thought to involve activation of the trigeminal afferents, which densely innervate dural structures and send projections to second-order neurons of

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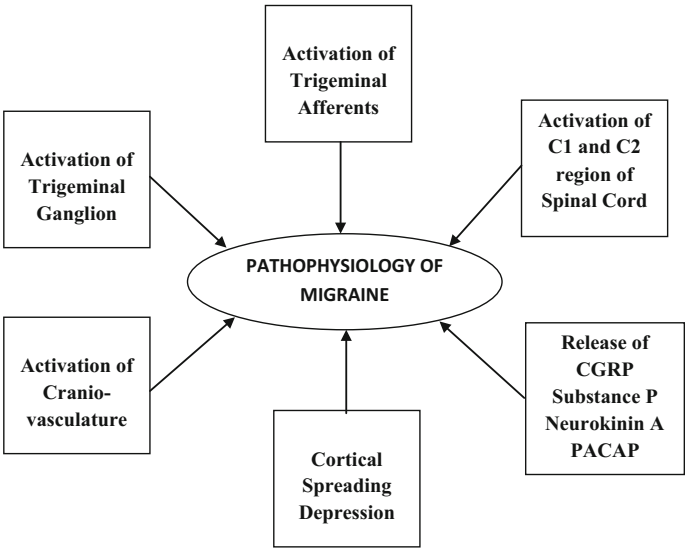

Fig. 1 Pathophysiology of migraine

trigeminal nucleus caudalis and C1–C2 region of the spinal cord (trigeminocervical complex). The trigeminovascular system includes the pseudounipolar trigeminal ganglion that projects into the trigeminal nucleus caudalis in the medullary spinal cord, and has a peripheral projection from the ophthalmic division of the trigeminal ganglion, which innervates the cranial blood vessels and pain-sensitive dura mater. Numerous potent vasodilator peptides including calcitonin gene-related peptide (CGRP), substance P, neurokinin A, and pituitary adenylate cyclase-activating peptide (PACAP) are present in the trigeminal ganglion and project nerve fibers to cranial vessels. Several lines of evidence suggest that cortical spreading depression (CSD) causes aura symptoms and impacts both short-term and long-term neurovascular function. It has been suggested that CSD is the first event identified upstream to trigeminovascular activation causing pain and characteristic blood flow changes. It has also been documented that migraine pain in humans arises due to the stimulation of blood vessels in dura mater. These points raise the concept of involvement of cerebrovascular system of dura mater in pathophysiology of migraine (Fig. 1).

1.1 Need of Animal Model

Further, it led to explosion in preclinical research of the nerve fibers that innervate the dural vasculature, and the likely involvement of the trigeminovascular system and the subsequent development of animal models of migraine. Over the past 2–3 decades, several animal models of migraine have been developed which helped out

to understand the pathophysiology of migraine, identify novel drug targets and drug treatments. These animal models have provided valuable knowledge and a skeleton to understand about the effect of hormones, chemicals, and various environmental factors like sound, light on migraine pathophysiology. Although most of the researchers have suggested that these animal models have lot of shortcomings, still some promising new drugs have been developed by utilization of these preclinical models. Animal models are extremely helpful in the understanding of brain disorders and in developing new therapeutic approaches. Migraine has recently become one of the major interests to neuroscientists. Various models for studying migraine headache mechanisms have been developed and exploited efficiently, leading to better understanding of the pathophysiology of the disorder and mechanism of action of anti-migraine drugs. These model systems have primarily focused on the pain-producing cranial structures, cerebrovascular system, and dura mater, in order to provide reproducible physiological measures that can be further subjected to pharmacological investigation. A wide range of techniques (both in vivo and in vitro approaches) are now in use, which include manipulation of the mouse genome to produce animal models having similarity with human disease.

2 Classification of Animal Models of Migraine (Fig. 2)

2.1 Acetic Acid-Induced Abdominal Constriction Test

Principle:

Acetic acid-induced writhing method or abdominal constriction is the best method used for the evaluation of analgesic activity. Writhing may be defined as a stretch or tension to one side, expansion of hind legs, and contraction of the abdomen in a

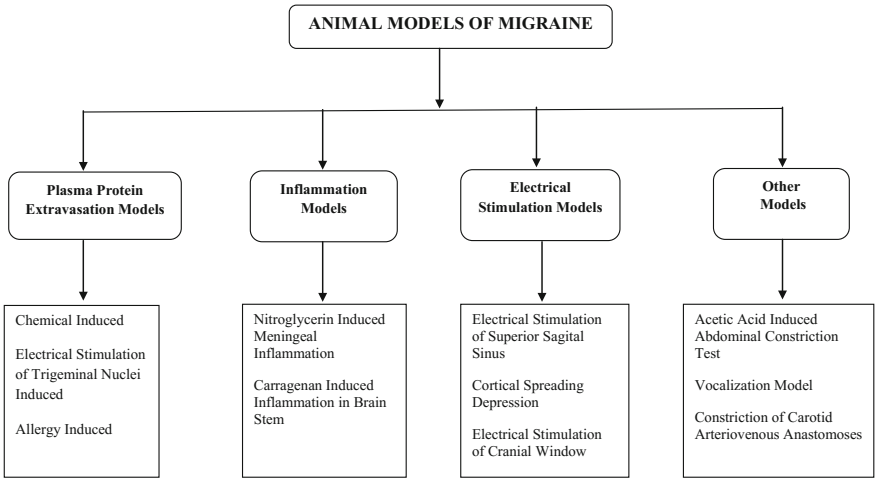

Fig. 2 Classification of animal models of migraine

way that the abdomen touches the surface and turning of trunk (twist). The anti-hyperalgesic activity of clinically used anti-migraine drugs like sumatriptan and ergotamine was first confirmed by using this model, and therefore, this test is also used as in vivo migraine model.

Procedure:

- Swiss albino mice (15–35 g) are used.
- 0.3% v/v solution of acetic acid is prepared in distilled water, and writhing is induced by the i.p. injection of 0.3% solution of acetic acid in volume of 0.1 ml/10 g body weight and is evaluated by counting the number of abdominal constrictions.
- In this model test and standard drug is administered before the acetic acid injection and number of abdominal constrictions are counted 5 min after acetic acid injection for a period of 10 min (Galeotti et al. 2002).

2.2 Nitroglycerin (GTN)-Induced Meningeal Inflammation

Principle:

Glyceryl trinitrate (GTN) infusion is the most widely used and trustworthy method to provoke migraine-like headaches in humans as well as in animals. The infusion of the nitric oxide donor nitroglycerin results in upregulation of proinflammatory mediators, macrophage activation, edema formation, and mast cell degranulation by its direct action on meningeal tissues and produces plasma protein extravasations (PPE). GTN is metabolized to nitric oxide (NO) by a combination of glutathione-S-transferase, cytochrome P450, and thiol reactions. Type II nitric oxide synthase (NOS) is expressed by macrophages and can be activated by GTN. After some hours of GTN infusion, iNOS generates NO at 100–1000 fold than its constitutive counterparts, and promotes inflammation and development of migraine. GTN activates second-order nociceptors, NF-κB, and increases the expression of c-fos, neuronal nitric oxide synthase (nNOS), and calmodulin-dependent protein kinase II in the trigeminal nucleus caudalis. Increased production of NO has been reported to upregulate COX, TNF-α, and matrix metalloprotease-9 (MMP-9).

Procedure:

- Sprague-Dawley rats (200–230 g) are used.
- Rats are infused with GTN (4 μg/kg/min, for 20 min, i.v.) a dose just 8–10 times higher than in humans (0.5 μg/kg/min, for 20 min, i.v.).
- Level of inflammatory mediators like TNF-α and mRNA and protein expression for c-fos is analyzed in the trigeminal vascular system at various time points using ELISA, RT-PCR, and immunohistochemistry.
- In other way, Sprague-Dawley rats are subcutaneously injected GTN (10 mg/kg) in the back of the neck and after 4 h rats are sacrificed for double

immunofluorescent labeling and Western blot analysis for the evaluation of nuclear NF-κB protein expression. This time point is chosen because previous studies have revealed the strongest nuclear NF-κB protein expression at this time (Bhandare et al. 2011).

Advantages:

1. It is the most widely used and reliable animal model for inducing migraine-like attacks in animals.
2. One of the most important advantages of the GTN-triggered headache model is the temporal control of events following GTN administration.

2.3 Vocalization Model in Rats

Principle:

Bradykinin (BK) is well implicated in microvasculature dilatation and in the generation of pain. It potently dilates cerebral arterial vessels and contributes to the vasodilatation, edema, and pain during migraine. BK has been reported as a possible pathogenetic factor of migraine. Taking this into consideration, the injection of a few micrograms of BK into a common carotid artery or into the cisterna magna of rabbits results in intense vocalization. Vocalization was also observed following the intra-carotid injection of BK into rabbits under general anesthesia (vocalization has long been accepted as a signal of pain in animals) (Martino et al. 2008).

Procedure:

- Wistar rats (200–250 g) are used, and BK is injected into the arterial catheter at the dose of 10 μg/kg in the volume of 10 μl/kg.
- Vocalization produced is recorded before, during, and for 5 min after BK injection with the help of microphone positioned at the snout of rat and is connected to the polygraph for recording (Ottani et al. 2004).

2.4 Plasma Protein Extravasations (PPE) Models

Principle:

Inflammation of the dura mater has been well implicated in the etiology of migraine; therefore, the blockage of PPE in the dura mater produced by trigeminal ganglion stimulation might play an important in anti-migraine activity of drug. ^{125}I-labeled bovine serum albumin or fluorescent isothiocyanate-bovine serum albumin (fluorescein) is used as marker for determining plasma protein extravasation. Plasma

extravasation and vasodilation are associated with inflammatory response developed by neurogenic or non-neurogenic mechanisms. Neurogenic inflammation accompanies electrical, mechanical, or chemical stimulation of sensory nerves fibers. Capsaicin, the active ingredient of hot peppers, activates sensory axons and causes the release of vasodilators and permeability-promoting transmitters from perivascular afferent axons and subsequent development of neurogenic inflammation. Non-neurogenic plasma extravasation can be induced by the i.v. administration of 5-HT, histamine, and bradykinin (BK), which directly alter blood vessel permeability.

2.4.1 Chemical-Induced PPE

Procedure:

- Anesthetized rats are injected with any of the following chemicals (capsaicin, neuropeptides, 5-HT, bradykinin (BK), histamine, or PGE2) at dose of 1 ml/kg into the femoral vein for the development of PPE.
- 15 min after injecting tracer and 10 min after drug injection, the thorax is perfused with saline via the left ventricle.
- The brain is removed and the dura is dissected and incubated, and the amount of marker in the dura is determined by any specific methods which give amount of PPE.

2.4.2 Electrical Stimulation-Induced PPE

Procedure:

- Anesthetized animals are placed in a stereotaxic apparatus, and holes of l–2 mm diameter are drilled on each side using following coordinates, i.e., intersection of the coronal and sagittal sutures: −1.2, 2.5, and −3.2, 2.5.
- Paired non-concentric bipolar electrodes are inserted to depth of 9.8–l0.2 mm below the surface of the calvarium, ensuring bilateral placement in the trigeminal ganglia. The left or right side is arbitrarily selected for stimulation.
- Paired rectangular pulses having opposite polarity are used to stimulate test side at the two different sites for 5 min with 5 pulses/set of 5 ms duration, at an intensity of 3.0 mA.
- The potential difference and current flowing across the electrode (20–30 V) are continuously monitored using an oscilloscope. Correct electrode placement is indicated by ipsilateral contraction of the muscles of mastication during stimulation.
- The brain is removed, and the dura is dissected. The dura is further incubated, and the amount of marker in the dura is determined by any specific methods which give amount of PPE.

2.4.3 Allergy-Induced PPE

Procedure:

- Guinea pigs are used and sensitized by a single i.p. injection of ovalbumin at dose of 1 μg and 100 mg $Al(OH)_3$, in 0.5 ml saline.
- After 4–6 weeks, animals are challenged with i.v. injection of 20 μg/kg ovalbumin.
- 10 min after ovalbumin challenge, the animals are perfused with saline via left ventricle.
- The brain is then removed, and dura is dissected. The dura is further incubated, and the amount of marker in the dura is determined by any specific methods which give amount of PPE.

2.5 Carrageenan-Induced Inflammation in Brain Stem

Principle:

Iatrogenic chemical stimulation of meningeal tissues is used to produce head pain. Carrageenan is a sulfated polysaccharide which promotes inflammation via activation of proinflammatory cells. It is widely used as algesic agent in numerous experimental studies. NO can induce headache in migraine patients and often triggers a delayed migraine. The initial component of headache results involves direct action of the NO-cyclic guanosine monophosphate pathway that causes vasodilatation and vascular smooth muscle relaxation, while the delayed component of headache involves trigeminovascular activation. It has been demonstrated that the increased nNOS activity in the trigeminal system causes CGRP release and dural vessel dilation. Also, it has been shown that intracisternal injection of carrageenan is associated with a significant increase in the NOS enzymes in the brain stem of rats.

Procedure:

- Wistar rats (200–250 g) are anesthetized and injected with single intracisternal injection of carrageenan into cisterna magna.
- The brain tissues are then removed and analyzed for endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) by immunohistochemistry (Bergerot et al. 2006).

2.6 Electrical Stimulation of Superior Sagittal Sinus

Principle:

The induction of trigeminovascular nociception with the help of electrical stimulation of the dura mater surrounding the superior sagittal sinus is widely accepted model for the examination of pathophysiology of vascular headache such as migraine. The

neurons of the trigeminal ganglion innervate the dural blood vessels in the periphery and trigeminal nucleus caudalis centrally (Dong et al. 2011). Pituitary adenylate cyclase-activating peptide (PACAP) is mainly present in human trigeminocervical complex and can trigger migraine. PACAP is a member of the vasoactive intestinal polypeptide/secretin/glucagon neuropeptide superfamily and acts on its specific receptor PAC1, and on two other receptors non-specifically, VPAC1 and VPAC2. PACAP may affect the paraventricular nucleus of the hypothalamus, and the hypothalamic region is well implicated in pathophysiology of migraine.

Procedure:

- Cats are anesthetized with an intraperitoneal injection of a-chloralose (70 mg/kg), with additional doses (20 mg/kg i.v.) during the experiment.
- Continuous measurement of blood pressure and heart rate is done by inserting catheters into the femoral artery and vein. A third catheter is inserted into jugular vein to permit sampling of blood.
- Cats are ventilated with 30% oxygen and are immobilized by i.v. administration of gallamine triethiodide (20 mg).
- Testing for sympathetic responses to noxious stimulation is conducted at regular intervals to assess the depth of anesthesia.
- The superior sagittal sinus is then stimulated with supramaximal square-wave stimulus-isolated shocks (100 V, 1 s duration, 10 Hz).
- Blood (5 mL) is to be taken before, and after, 7–8 min of stimulation and the volume of blood is replaced by an equivalent amount of plasma expander.

2.7 CSD Model of Migraine Aura

Principle:

Cortical spreading depression (CSD), which is known as shift in cortical steady potential, results in an increase in extracellular ions and neurotransmitter like glutamate, and sustained increase in cortical blood flow followed by transient decrease in blood flow, in occipital lobe and is responsible for generation of aura before the migraine attack. These ions and neurotransmitters cause intense vasodilatation in the cortex, pial vessel, and dura mater, and activate the trigeminal afferents and transmit impulse to the trigeminal ganglia and trigeminal nucleus caudalis (TNC) which is involved in the processing of pain. CSD is an intense depolarization of neuronal and glial membranes accompanied by a massive disruption of ionic gradients and loss of membrane resistance (Charles et al. 2013).

Procedure:

- Male Sprague-Dawley rats are anesthetized with urethane (1.8 g/kg; i.p.) and placed on a stereotaxic frame. Core temperature is maintained at 37 °C using a heating blanket.

- The dura over the left side is exposed between the bregma and 2 mm caudal to lambda. The exposed dura is kept moist throughout the experiment, with the help of modified synthetic interstitial fluid having pH 7.2. The fluid is composed of 135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES.
- Single waves of CSD are induced using mechanical, electrical, and chemical stimulation of the visual cortex, approx 6 mm away from the dural receptive field of the neuron under study.
- Changes in steady potential on the surface of the cortex are recorded using a glass micropipette (containing 50 mM NaCl), between the dural receptive field and the site of cortical stimulation. At a propagation rate of 3–5 mm/min, a wave of CSD enters the neuronal receptive field within 1–2 min of cortical stimulation.
- Mechanical stimulation (pin prick) is given by inserting a glass micropipette (diameter 25 μm) 1 mm into the visual cortex for 10 s.
- Electrical stimulation (cathodal pulses) is given by using a concentric bipolar electrode after every 90 min until a wave of CSD is recorded (current—0.5 or 1.0 mA; duration—1 or 3 ms; frequency—between 0.2 and 50 Hz.

2.8 Constriction of Carotid Arteriovenous Anastomoses in Anesthetised Animals

Principle:

It has been suggested that the arteriovenous oxygen difference during migraine is reduced. This finding, when corelated with clinical symptoms such as facial paleness, reduction in facial temperature, increased pulsations in temporal artery and inflammation of the frontal vein on the side of the headache lead to the implication that arteriovenous anastomoses are involved in the pathogenesis of migraine (De Vries et al. 1998).

Procedure:

- In conscious pigs, constriction of arteriovenous anastomoses is done under strong pressure of the sympathetic neuronal tone, which shunts 3% part of the total carotid blood flow.
- In contrast, under pentobarbital anesthesia, 80% of the total carotid blood flow in the pig is limited via arteriovenous anastomoses into the jugular vein.
- Radioactive microspheres are used to measure the carotid blood flow and the effects of anti-migraine drugs on this parameter.

2.9 Electrical Stimulation of Cranial Window

Principle:

One hypothesis is that nerve fibers within the trigeminal nucleus get activated during migraine headache resulting in the release of vasoactive peptides and the consequent dilation of meningeal blood vessels. Electrical stimulation of the cranial window results in activation of the trigeminal nerves via the release of CGRP from presynaptic trigeminal nerve endings ultimately leading to the dural and pial blood vessel dilation. The inhibition of dural vasodilation proved to be successful in predicting anti-migraine efficacy of triptans, 5-HT1B/1D receptor agonists, dihydroergotamine, and CGRP receptor antagonist.

Procedure:

- Male Dunkin Hartley guinea pigs (300 ± 45 g) are anesthetized throughout experiments with pentobarbitone sodium (initially 50 mg/kg i.p., then 18 mg/kg, i.v., constant infusion).
- The trachea, left carotid artery, and jugular vein are cannulated for artificial ventilation, measurement of mean arterial blood pressure (MABP), and intravenous injection of anesthetic and drugs, respectively.
- Guinea pigs are placed in a stereotaxic frame, the skull exposed and the right parietal bone is drilled until the dural blood vessels becomes clearly visible through the intact skull.
- The dural blood vessel diameter is continuously measured by a video dimension analyzer.
- In preliminary experiments, it has been observed that the dural blood vessels are observed to be maximally dilated, so that electrical stimulation of the cranial window will produce very less increase in diameter. It is therefore necessary to preconstrict the dural vessels with i.v. administration of endothelin-1 (3 mg/kg).
- After single administration of endothelin-1 (3 mg/kg, i.v.) dural vasodilation is induced approximately 3 min later by i.v. CGRP (1 mg/kg) or electrical stimulation of the cranial window (250 ± 300 mA, 5-Hz, 1 ms to 10 s) and is expressed as percentage increase in dural blood vessel diameter + SEM, from baseline (Williamson et al. 2001).

3 Conclusion

Migraine is a mysterious disorder characterized by chronic headache and is often associated with nausea, vomiting, sensitivity to light and sound. Different drugs are available to treat migraine attack, but need for novel effective therapy is insistent. Different animal models described in this chapter can be used to explore the pathophysiology of migraine and to test novel drugs. Every model described

reflects some particular pathological features of the migraine like vasodilation, plasma protein extravasation, and cortical spreading depression. A specific model can be picked to induce the disease on the basis of expected mechanism of action of new drug.

Ethical Statement:

All institutional guidelines, national guidelines, state and local laws, and regulations with professional standards for the care and use of laboratory animals should be followed. Studies involving animals must state that the institutional animal ethical committee has approved the protocol. For authors using experimental animals, a statement should be made that the animals' care is in accordance with institutional guidelines and animals used have been treated humanely and with regard for the alleviation of suffering. Researchers should treat animals as sentient and must consider their proper care and use and the avoidance or minimization of discomfort, distress, or pain as imperatives. Animal experiments should be designed only after due consideration of animal health. It should be ensured that all researchers who are using animals have received instruction in research methods and in the care, maintenance, and handling of the species being used. All the surgical procedures should be performed under appropriate anesthesia and follow only those procedures which avoid infection and minimize pain during and after surgery.

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