CHAPTER II

REVIEW LITERATURE

I. MIGRAINE

DEFINITION AND CLASSIFICATION

Migraine is a primary neurobiologic disorder, resulting from dysfunction of the trigeminovascular system. The disorder manifests as recurring attacks, usually lasting 4-72 hours. These attacks, which can interfere with normal functioning, involve unilateral throbbing headache pain of moderate to severe intensity. They also usually involve nausea, sometimes vomiting, and light, sound, and sensitivity to other sensory stimuli (Blau, 1992).

Migraine may occur with or without an aura that, when present, generally lasts between 5 and 60 minutes. Aura occurs in about 15-20% of patients with migraine. The most common type of aura is visual but also can be somatosensory. The typical presentation of the visual aura is the scintillating scotoma, also referred to as "fortication specta" or "teichopsia" (Figure 2.1).

In most patients who experience aura, the aura develops before the head pain begins, but on occasion an aura may appear or recur when the headache is most intense. An aura is present before every migraine attack in some individuals, but in other patients, aura accompanies only a small proportion of attacks. The intensity of aura varies among attacks and may remain constant from attack to attack in a particular person or may vary in successive attacks in the same person. However, aura only occurs in a minority of patients with migraine.

Furthermore, premonitory symptoms, called prodrome, may precede migraine attack; these symptoms occur 24-72 hours before the onset of other symptoms. During this period, patients may experience feelings of well-being, talkativeness, surges of energy, hunger, anorexia, drowsiness, excessive yawning, depression, irritability, restlessness, or tension.

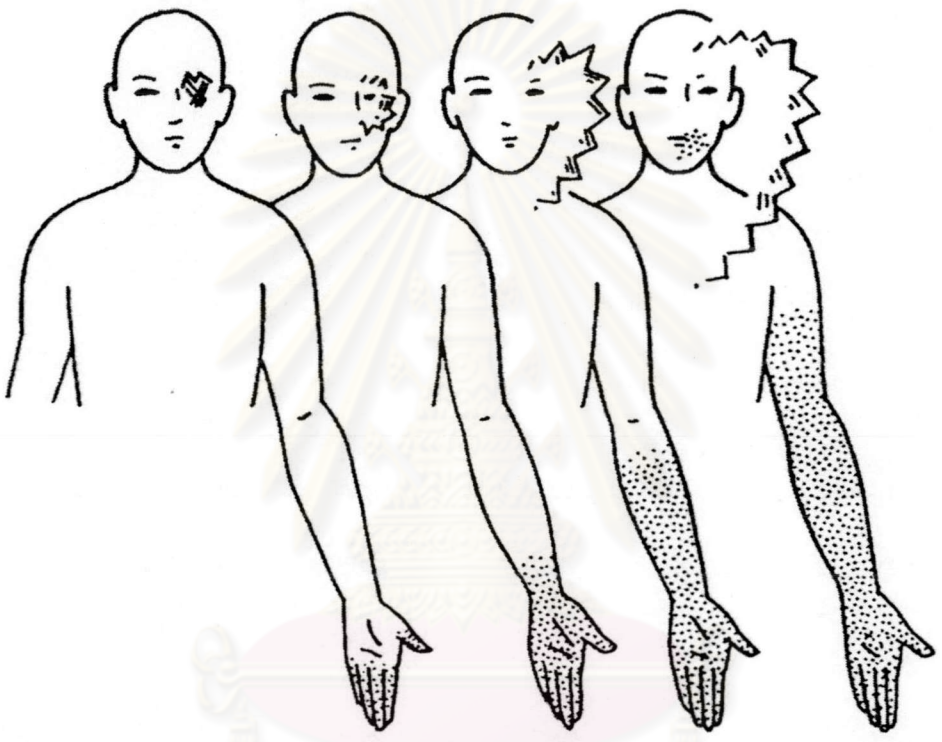

Figure 2.1 *Scintillating scotoma (above) and digitolingual syndrome (below) from left to right in their successive stages of development.*

CLASSIFICATION OF MIGRAINE

Migraine headaches are classified according to their clinical features, as well as according to current concepts of pathophysiology. Patients who have migraine without aura generally have normal cerebral blood flow and do not report focal neurologic symptoms. In those who have migraine with aura, changes in regional cerebral blood flow can be

demonstrated and neurologic symptoms originating in the brain or brain stem are reported (Lauritzen and Olesen, 1984).

The current IHS classification system recognizes several subtypes of migraine with aura and several other specific types of migraine (Table 2.1).

Migraine without aura

Migraine without aura is an idiopathic recurring headache disorder that manifests in the form of attacks that last 4-72 hours. Typically, the headaches are unilateral, have a pulsating quality, and are moderate to severe in intensity. These headaches are aggravated by routine physical activity and are associated with nausea or vomiting, photophobia, and phonophobia.

Migraine with aura

Migraine with aura is also an idiopathic recurring disorder that manifests in some women as migraine without aura but is also accompanied by transient neurologic symptoms. Aura symptoms usually develop gradually, over 5-20 minutes, and last less than 60 minutes. Headache, nausea, or photophobia usually followed neurologic aura symptoms directly or after a free interval of less than 1 hour. The headache usually lasts 4-72 hours, but rarely will be completely absent (migraine aura without headache).

The visual aura symptoms

The visual disturbance usually precedes the headache. It begins near the center of the visual field as a small gray area with indefinite boundaries. If this area first appears during reading, as it often does, then the migraine is first noticed when words are lost in a region of "shaded darkness." During the next few minutes the gray area slowly expands into

a horseshoe with bright zigzag lines appearing at the expanding outer edge. These lines are small at first and grow as the blind area expands and moves outward toward the periphery of the visual field.

One important aspect of the visual disturbance just described, is that it expands slowly, over a period of 10 to 20 minutes. The initial region of visual abnormality is most often near fixation and then, as described by Lashley, with increase in size the disturbed area moves or "drifts" across the visual field so that its central margin withdraws from the macular region as its peripheral margin invades the temporal, the area may be totally blind (negative scotoma), amblyopic or outlined by scintillations.

The scintillations surrounding the negative scotoma make "fortification" figures or spectrums. The scintillations are brilliant, with the intensity of a bright fluorescent bulb flickering at a rate of 5 to 10 cycles/second. Illustrations are portrayed in figure 2.2.

Figure 2.2 *Successive maps of a scintillating scotoma to show characteristic distribution of the fortification figures. In each case the asterisk indicates the fixation point. Knowledge of the retinotopic organization of the visual cortex allowed Lashley (1941) to calculate the speed of propagation of the excitation-depression wave as ~3 mm/min.*

Not all migraine visual disturbances begin near the fixation point. Some patients consistently experience scotomas starting eccentrically in the visual field, and these sensations can appear alternately or simultaneously in both hemifields.

The duration of these visual symptoms is measured in minutes rather than the brief few seconds of flashing, bright moving spots. Variations in the scotomas of migraine, including their occurrence in patients with acquired blindness, are well described.

Other types of migraine

Ophthalmoplegic migraine, an extremely rare condition that may affect the ocular nerves, is characterized by drooping of the eyelids and dysfunction of the extraocular muscles those results in double vision. Another rare form of migraine is retinal migraine, in which inadequate blood supply to the retina results in a scotoma or transient blindness affecting one eye. This differs from the visual disturbances usually associated with migraine aura in that only one eye is affected in retinal migraine compared with the visual disturbances being perceived in both eyes in migraine with visual aura.

Although not all of these disorders have been classified, the IHS classification of headache disorders lists two such syndromes along with diagnostic criteria.

Table 2.1 Classification and Characteristics of Migraine According to the Criteria Set by the International Headache Society.

Migraine without aura: idiopathic, chronic headache disorder occurring in attacks.

1. Headache attacks lasting 4-72 hours with pain-free intervals between headaches
2. Pain characteristics (at least two necessary):
    a. Unilateral location
    b. Pulsating quality
    c. Moderate or severe intensity
    d. Aggravation by physical activity
3. Symptoms during the headache (at least one required):
    a. Nausea and/or vomiting
    b. Photophobia and/or phonophobia
4. At least one of the following:
    a. No organic neurological disorder found on history, physical, and neurological examinations
    b. History, physical, or neurological examination suggests organic disorder, but neuroimaging or laboratory procedures rule out the possibility
5. The patient must have at least 5 attacks fulfilling criteria 1 to 4 above

Migraine with aura

1. Patient must fulfill criteria 1-5 above.
2. Patient must have at least three of the following four characteristics with a headache:
    a. One or more fully reversible symptoms that are manifestation of focal hemispheric and/or brain stem dysfunction
    b. At least one aura symptom develops gradually over 4 minutes or more or symptoms may occur in succession
    c. Aura symptoms last less than 60 minutes
    d. Headache follows the aura by an interval of less than 60 minutes but may occasionally begin before the aura
3. The patient has at least two attacks fulfilling criterion 1 above

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Source: Headache Classification Committee, 1988.

PATHOPHYSIOLOGY OF MIGRAINE

Several theories on the pathogenesis of migraine exist. A clinically relevant model of migraine pathophysiology may explain several phenomena that both clinicians and patients have noted:

- Dietary, sleep, and hormonal changes can trigger a migraine.
- Migraine has a circadian rhythm similar to several diseases of vasoconstriction, such as myocardial infarction, angina pectoris, and ischemic stroke.
- Sleep is effective in aborting many migraines.
- Cerebral blood flow is decreased during migraine aura.
- Both generalized systemic vasoconstriction and local cerebrovascular vasodilation occur on the side where head pain occurs.
- Platelets release serotonin during a migraine attack.
- Levels of calcitonin gene-related peptide (CGRP), and probably substance P, are elevated during a migraine attack.

Common Theories of Migraine Pathogenesis

Theories on the pathogenesis of migraine include:

- the vascular theory
- the cortical spreading depression theory
- the neurovascular hypothesis
- the serotonergic abnormalities hypothesis
- the integrated hypothesis.

The vascular theory

Wolff developed the vascular theory of migraine pathogenesis during the 1940s and 1950s. According to this theory, migraine is a vasospastic disorder that is initiated by vasoconstriction in the cranial vasculature (Wolf, 1981). The vasoconstriction stage appears to be associated with migraine aura.

Following the early vasoconstrictive stage, intracranial or extracranial blood vessels dilate. Whereas most of the brain is insensitive to pain, meningeal blood vessels show a high level of innervation. Thus, blood vessel dilation activates the trigeminal sensory nerves that surround the meningeal blood vessels, causing pain (O' Connor et al., 1986). Activation of trigeminal nerves also causes the release of vasoactive neuropeptides that further contribute to dilation and worsen pain (Markowitz et al., 1987).

Studies have documented the occurrence of oligaemia during the aura phase of a migraine, and an increase in blood flow during the headache phase. Moreover, when a patient with a headache is given a vasodilator such as a nitrate, the headache intensifies, whereas when a patient is given a vasoconstrictor such as a 5-HT agonist, the headache is usually alleviated (Friberg et al., 1991). These studies lend support to the vascular theory.

However, some researchers have questioned whether the measured decreases in cerebral blood flow during the aura phase are sufficient to cause the aura symptoms (e.g., visual disturbances) that some migraineurs experience. Furthermore, vasodilation alone cannot explain the local swelling and tenderness of the head that generally accompany migraine.

The cortical spreading depression theory

Cortical spreading depression (CSD) is a relatively short-lasting wave of depolarization that spreads across the surface of the brain, moving from the back (occipital region) of the cerebral cortex toward the front at about 3-5 mm/minute (Figure 2.3). This electrical phenomenon can be induced in animals with non-noxious stimuli, and is frequently referred to in the literature as the "spreading depression of Leao" (Lauritzen, 1994).

Cortical spreading depression: basic features

The reaction was first identified in rabbit cerebral cortex (Leao, 1944a,b). The basic observation was that the EEG following mild noxious stimuli would become completely extinguished for a minute or so and that the depression would propagate very slowly across a wide cortical region.

Cortical spreading depression has been induced in most grey matter regions (Bures et al., 1974). It has been observed in human cortical tissue in vitro (Avoli et al., 1991), and in human hippocampus and striatum in vivo (Sramka et al., 1978). Thus, human cortical tissues do support the development of CSD, but a recording of CSD from the human neocortex in vivo is still missing (Gloor, 1986).

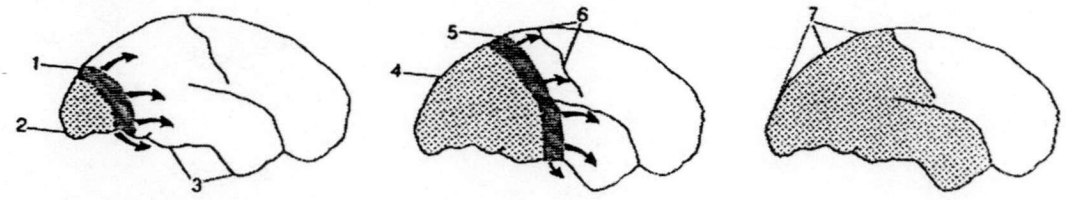

Figure 2.3 *Hypothesis of development of a migraine attack based on aspects of CSD and migraine. The figures represent lateral views of the human brain at different time intervals after the start of the attack, spaced by 30 min. The dotted area represents the region of reduced rCBF, the striped area represent the region of neuronal depolarization during the first minute of CSD. 1, Initially during a migraine attack a CSD is elicited at the occipital pole, spreading anteriorly at the lateral, medial, and ventral sides of the brain. At the CSD wave front, transient ionic and metabolic disequilibria trigger perturbed neuronal function, rCBF changes and neurological symptoms. 2, Following CSD, cortical rCBF decreases by 20-30% for 2-6 hours. 3, rCBF in regions not invaded by CSD remains normal until encountered by CSD. 4, The region of reduced rCBF expands as the CSD moves anteriorly. 5, Somatosensory symptoms from the extremities appear when the CSD invades the primary sensory cortex at the post-central gyrus. 6, CSD usually stop on reaching the central sulcus, but in many patients it does not even propagate this far. The ventral spread of CSD causes activation of pain-sensitive fibers and headache. 7, Full-scale attack. The CSD has stopped and is now detectable as a persistent reduction of cortical rCBF. At this time the patient suffers from headache, but has no focal deficits.*

Successful elicitation of CSD in experiments depends on the susceptibility of the tissue and the trigger factor involved. Common methods of triggering CSD include local electrical or mechanical stimulation or injections of high concentrations of KCl. Potassium plays a central role for CSD and it is reasonable to assume that any disturbance of K^+ homeostasis would predispose the brain region to CSD (Grafstein, 1963). Brain K^+ clearance systems are heavily dependent on the capacity of glial cells (Nicholson and Kraig, 1981). In humans the lowest glial-neuronal cell ratio is in the primary visual cortex. Therefore, one would expect human CSD to be initiated occipitally. As is well known, visual auras are indeed very frequent in migraine (Olesen et al., 1990).

Neurons and glial cells depolarize during CSD, giving rise to an intense, but transient spike activity when the reaction enters the tissue (Sugaya et al., 1975). Neuronal silence immediately follows, lasting for a few minutes, but evoked potentials usually take a longer time to recover, 15-30 min (Bures et al., 1974). This sequence of brief excitation followed by a short-lasting depression is supposed to be the neurophysiological basis of the sensory symptoms during migraine auras (Leao and Morison, 1945; Milner, 1958; Gardner-Medwin, 1981; Lord, 1986; Lauritzen, 1987a,b).

The depolarization is associated with dramatic changes in the distribution of ions between the intra- and extracellular compartments: K^+ and hydrogen ions leaves the cells, while Na^+, Ca^{2+} and Cl^- enters together with water, as the size of the extracellular space decreases to approximately half of the control values (Figure 2.4) (Nicholson and Kraig, 1981; Hansen, 1985). A return to normal of most ion concentrations and of the size of the extracellular space occur spontaneously after 30-60s, whereas Ca^{2+} and pH usually take a few more

minutes to recover. There is no satisfactory explanation of the spreading mechanism of CSD, but the spread probably involves the diffusion of one or more chemical mediators, most likely K^+ and glutamate, into the extracellular compartment (Nicholson, 1993). It has been suggested that a calcium wave in glial cells underlies CSD, but this still remains to be proven (Leibowitz, 1992).

A simplistic scheme of the mechanism of spread of CSD is given in figure 2.5. It is important to appreciate the transient nature of CSD. If the electrophysiological changes are sustained and propagation is absent, then the phenomenon is usually anoxia or hypoglycaemia rather than CSD. Repeated episodes of CSD increases the immunohistochemical staining of glial fibrillary acidic protein in the rat cortex that is associated with activation of this cell type (Kraig et al., 1991) and a prolonged period (24 h) of expression of the c-fos proto-oncogene (Herrera et al., 1993) and inhibition of protein synthesis (Mies, 1993).

Cortical spreading depression phenomena occur in experimental animals in the penumbra zone, immediately adjacent to a cortical infarct, where nerve cells are viable but electrically silent (Nedergaard and Astrup, 1986; Gill et al., 1992; Ijima et al., 1992). In many respects, the ionic disequilibria during CSD resemble transient ischemia, but there is usually no shortage of energy supply during CSD (Lauritzen et al., 1990). These dramatic changes of neuronal function and ion homeostasis are associated with profound changes of the local circulation (Figure 2.6).

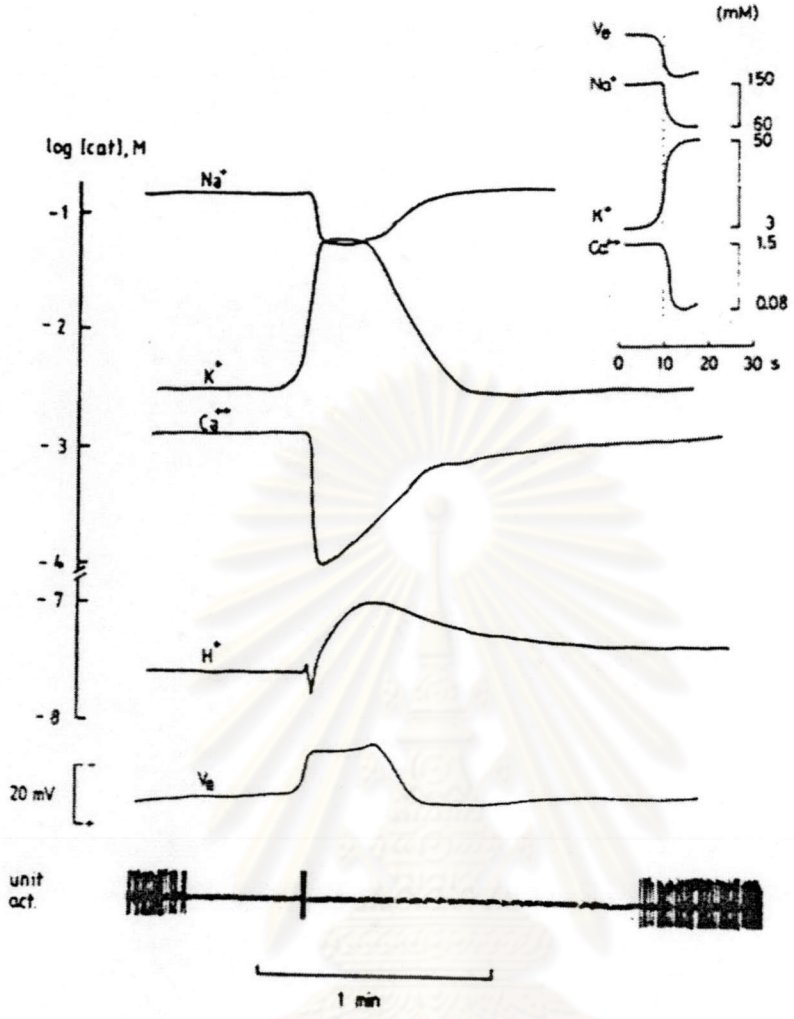

Figure 2.4 *Electrophysiological changes accompanying cortical spreading depression in rat brain. Interstitial ion concentrations of sodium, potassium, calcium and hydrogen were measured by ion-selective electrodes. The extracellular potential (V_e) and the single unit activity were measured by single-barrelled potential electrodes. Cortical spreading depression was elicited in frontal cortex and the electrophysiological changes were recorded in the parietal cortex.*

Reaction-diffusion model of cortical spreading depression

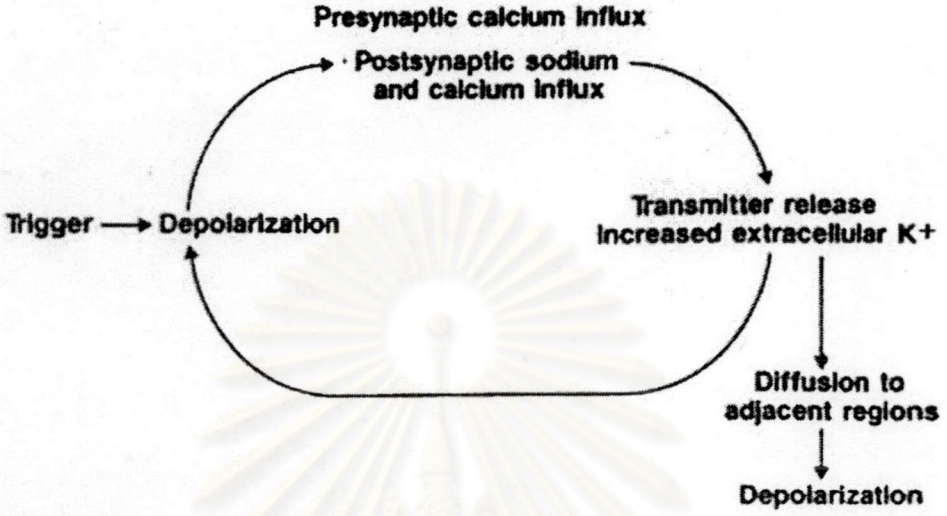

Figure 2.5 *Simplistic scheme of autocatalytic cycle possibly occurring during CSD.*

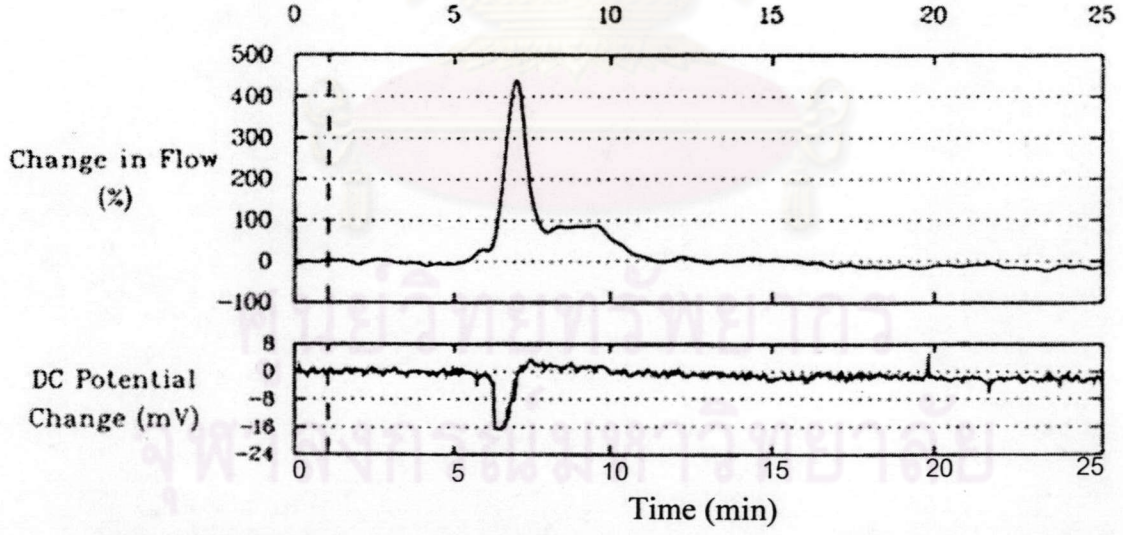

Figure 2.6 *typical tracing showing blood flow changes occurring after CSD which was initiated by pinprick 10 mm from the site of laser Doppler probe. DC potential was recorded from an electrode placed under probe (Piper et al., 1991).*

Technique for induction of CSD in animals

Several methods have been employed to initiate CSD in animal brain. These methods can be grouped into 3 major categories including local electrical, mechanical or chemical stimulations. Among these methods two techniques which are frequently used are mechanical stimulation and KCl application.

Mechanical stimulation

Blunt stabbing and pin pricking are the common method to elicit CSD. Several research groups, such as Lambert et al and Ebersberger et al always choose the pinprick technique to evoke CSD on the animal cortex, including cats, rats and rabbits (Lambert et al., 1999; Lambert and Michalicek, 1994; Piper et al., 1991; Ebersberger et al., 2001). This method is delivered by rapid insertion of a 26-30 gauge needle, through the dipole and dura (1-2 mm depth) into cortical tissue and immediately withdrawn. The location needs avoiding the pial vessels. However, this technique has been disagreed with some research groups. They suggested that this technique may link to the noxious stimulation (Read et al., 2000).

KCl application

As previously mentioned, potassium play a central role for CSD and it is reasonable to assume that any disturbances of K^+ homeostasis would predispose the brain region to CSD (Grafstein, 1963). The KCl application is the most popular technique that has been chosen by many research groups, such as Read et al, Jander et al, Smith et al, Moskowitz et al and Colonna et al. This technique can be further divided into 2 methods, including the microinjection of KCl on the cortical surface and the placement of solid KCl on the surface of cortex.

- The microinjection of KCl technique

After performing craniotomy, a fused silica needle or PE-90 (a catheter tube) or glass micropipette is placed ~1 mm below the dural surface. Then KCl is injected to the cortical area. The concentrations of KCl vary 670 mM to 1 M. (Ingvardsen et al., 1997; Meng et al., 1995; Moskowitz et al., 1993b; Colonna et al., 1997)

- The placement of solid KCl technique

After the craniotomy opening is performed, a solid KCl is placed directly to the cortical surface area. The weights of KCl vary 3 to 30 mg, depending on the size of animal (for example, rat uses 3 mg and cat uses 30 mg). (Smith et al., 2000; Read et al., 1997a,b; Read and Parsons, 2000; Read et al., 2000)

Less popular methods of KCl application are able available, such as a remote intracortical injection, application of KCl-soaked paper on cortical surface, etc. (Wahl et al., 1994; Douen et al., 2000; Koponen et al., 1999)

For the sake of comparison, application of NaCl into brain tissue is usually included as the control.

Cerebral blood flow during CSD and migraine

Cerebral blood flow during and after spreading depression in rats has been studied by autoradiographic methods (Lauritzen et al., 1982). These studies demonstrated that cortical blood flow is reduced by 20 to 25 per cent following induced CSD. However, CSD has been shown to induce transient vasodilatation of the pial circulation in a number of anesthetized animals (Wahl et al., 1994). Although CSD has been demonstrated only in animals, support for the CSD theory comes from observations that, in patients who have migraine with aura, a gradual spread of reduced blood flow that mimics the rate of progression of CSD in animals can be measured during the aura phase.

This CSD hypothesis is supported by the clinical observation of slowly spreading symptoms in migraine with aura by Olesen et al in 1981 (Olesen et al., 1981a). They studied regional cerebral blood flow (rCBF) changes in patients during a classic migraine attack. A gradual spread of reduced blood flow was observed starting in the occipital region and advancing anteriorly. The rCBF measurements showing gradually enlargement of hypoperfused region in migraine patients resembles that of a CSD seen in the animal brain. Olesen et al (1987) speculated that the aura of classic migraine may be occurred secondary to the spreading oligaemia observed in classic migraine patients. This theory states that migraine results from an evolving process in the cerebral cortex that occurs secondarily to decrease cortical function decreased cortical metabolism, and/or vasoconstriction of cortical arterioles.

In contrast to the blood flow changes reported during attacks of migraine with aura, regional oligaemia has not been observed in patients suffering from migraine without aura. Lauritzen and Olesen (1984)

studied 12 patients within 20 hours after the onset of a migraine without aura. There were no changes in focal or global cerebral blood flow in any of the patients. In addition, Olesen et al (1981b) studied 12 patients in whom attacks could be provoked by red wine. In studies of patients in whom migraine could be induced, rCBF were within normal limits. Thus, rCBF appears to be normal during a migraine without aura attack. This may be summarized that focal rCBF changes are related to the aura symptoms, but not related to the throbbing headache and other associated symptoms that are similar in both types of migraine.

Transmitters releasing during CSD

Indeed, the brain cortex releases excitatory amino acids, including glutamate and aspartate, to the interstitial fluid during CSD, but the increase is brief, lasting for only ~1 min (Figure 2.7) (Fabricius et al., 1993). CSD is blocked by various competitive and non-competitive NMDA antagonists but not by antagonists of non-NMDA glutamate subtype receptors (Van Harreveld, 1984; Gorelova et al., 1987; Lauritzen and Hansen, 1992). NMDA receptors activation in turn triggers the synthesis of NO, which are both an important vasodilator and a neurotransmitter. Furthermore, Read et al (1997a,b) demonstrated that the NO level was increased during CSD (Figure 2.8). This is of interest since nitroglycerin provokes headache in man (Iversen et at., 1989a,b).

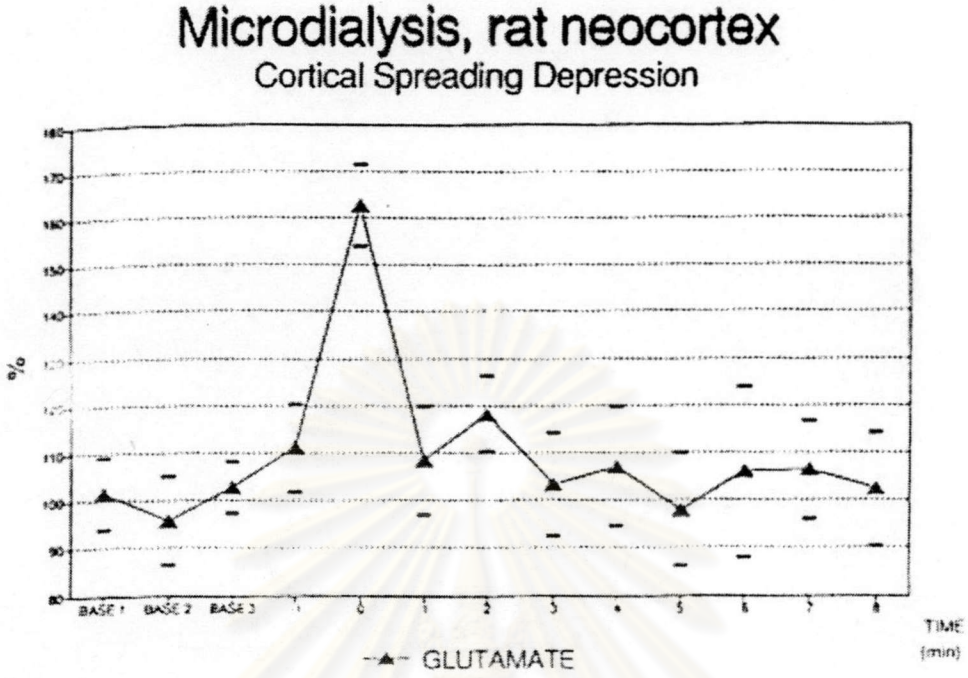

Figure 2.7 *Maximal concentrations in dialysate of glutamate during single episodes of CSD of rat brain. Graphs represent mean values = 1 SD of eight experiments at different time points during CSD. The CSD was elicited by needle stab in the frontal cortex while the dialysate was from the parietal cortex. Time zero corresponds to maximum of negative DC potential recorded with a single-barelled micro-electrode placed adjacent to the microdialysis probe. Amino acid concentrations were determined by HPLC (From Fabricius et al., 1993).*

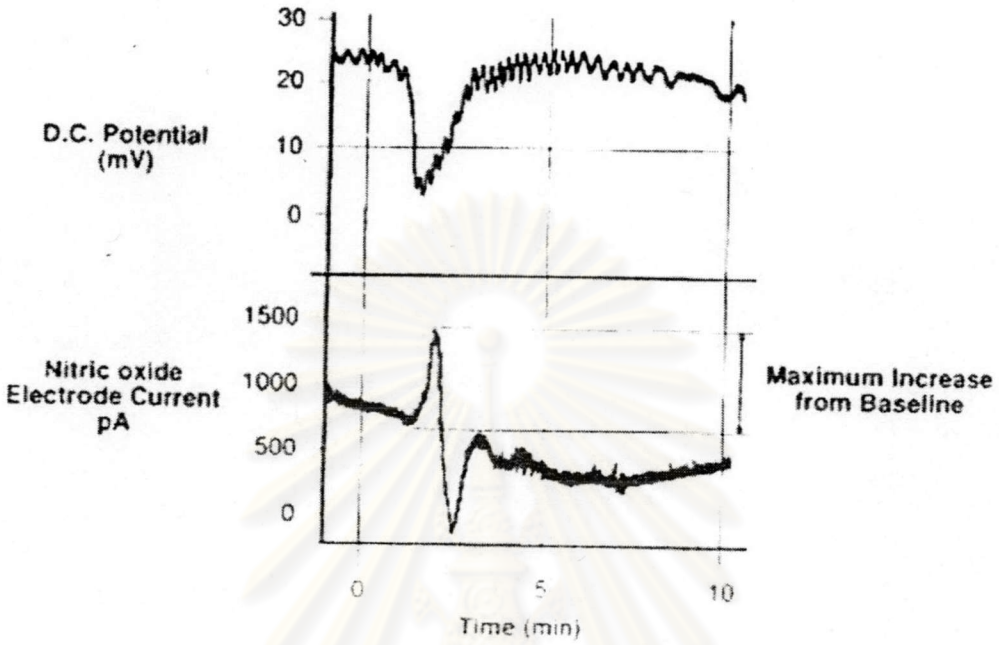

Figure 2.8 *Example trace of changes in cortical D.C. potential (mV) and NO electrode current (pA) during KCl evoked repeated CSD (Read et al., 1997)*

CSD as a migraine mechanism: the theory

The generation of headache from intracranial sources requires activation of pain-sensitive fibers that are located at the ventral surface of the brain. Recent data suggest that CSD activates nociceptive fibers in rats (Moskowitz et al., 1993b).The aura and headache may represent separate effects of CSD on different brain structures the cortex and the nociceptive vascular afferents. This may explain the variations of migraine symptoms observed in the same patient and also in different patients.

Moskowitz et al (1993b) suggested that neurophysiological events within cerebral cortex can activate brainstem regions involved in the processing of nociceptive information via trigeminovascular mechanisms (Figure 2.9). In this study, the effects of CSD were examined on the expression of immunoreactive Fos protein within the superficial lamina of trigeminal nucleus caudalis (TNC). CSD was induced by KCl application on the cortical cortex. In response, Fos-like protein was visualized in the ventrolateral TNC (corresponding to the ophthalmic division), chiefly in lamina I, IIo and predominantly within spinal segment. CSD significantly increased cell staining within ipsilateral TNC. Fos immunoreactivity staining was reduced after chronic surgical transection of meningeal afferents and recurrent CSD. Pretreatment with sumatriptan attenuated Fos like-protein in this model as well.

CSD may be one of several neurophysiological events capable of activating nociceptive mechanisms in cerebral cortex. Generalized seizures activate trigeminovascular fibers, and in so doing, increase blood flow in neocortex (Sakas et al., 1987).

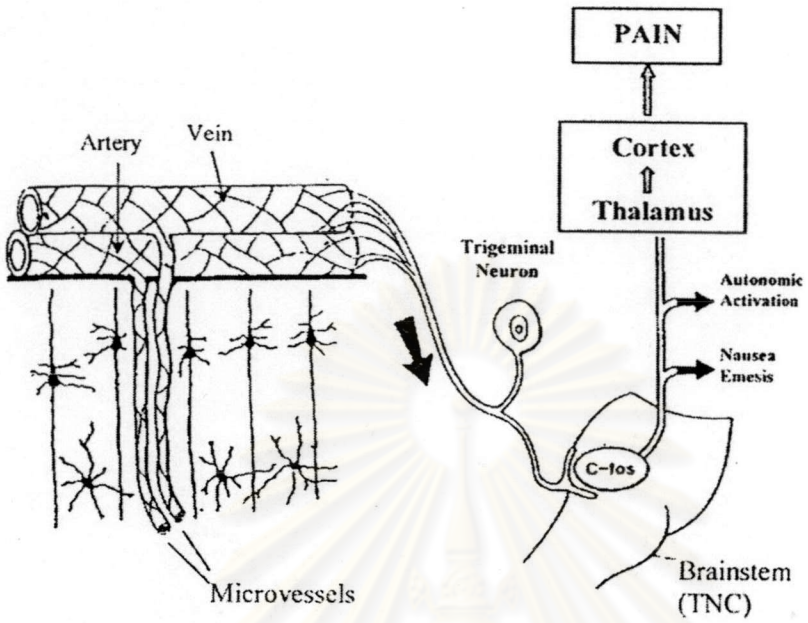

Figure 2.9 *Diagram depicting the relationship between pial vessels, the trigeminovascular system and neocortex. Recent data indicate that neurophysiological activity within neocortex (i.e. recurrent spreading depression) can activate the ipsilateral trigeminovascular system, as evidenced by induction of the c-fos antigen within lamina I, IIo of trigeminal nucleus caudalis. One postulation holds that ions, neurotransmitters, and other biologically active substances released into the extracellular space from neurons and glia may activate innervating fibers after reaching the perivascular space. Of note is that sectioning the trigeminal branch innervating the meninges or systemic administration with sumatriptan can significantly reduce the c-fos response. (From Moskowitz, 1993b)*

Magnetic resonance spectroscopy has revealed low brain magnesium in migraine patients (Swanson, 1988), a condition that strongly facilitates the elicitation of CSD in turtle, rat and human brain tissue in vitro (Mody et al., 1987; Lauritzen et al., 1988; Avoli et al., 1991). Plasma levels of glutamate and aspartate are elevated in migraine patients suggesting impairment of the amino acid reuptake mechanisms (Ferrari et al., 1990). Glutamate metabolism in migraine patients appears to be a fruitful area for future migraine research.

Drugs used for prophylactic migraine treatment including methysergide, propanolol, pizotifen, clonidine or flunarizine are ineffective as blockers of CSD (Hansen et al., 1984; Marranes et al., 1986). Probably these substances influence the sequence of events which cause pain (Moskowitz, 1992) rather than the CSD itself. Sumatriptan, the newly developed drug for treatment of migraine headaches, decreases the cortical input to the TNC of the brainstem without affecting the CSD itself (Moskowitz et al., 1993b). Ergotamine on the other hand increases the threshold for CSD in rats (Marranes et al., 1986).

According to the theory, CSD is associated with disturbances in nerve cell metabolism and regional changes in blood flow. Furthermore, CSD is considered play an important role in the pathophysiology of migraine with aura and some transmitters releasing during CSD may involve in this mechanism.

The neurovascular hypothesis

Fibers from the trigeminal nerve innervate blood vessels in the meninges, the extracranial arteries, and those in the circle of Willis. These nerve fibers contain nociceptors that are capable of generating pain impulses, and the endings of these nerve fibers contain peptide neurotransmitters (Figure 2.10).

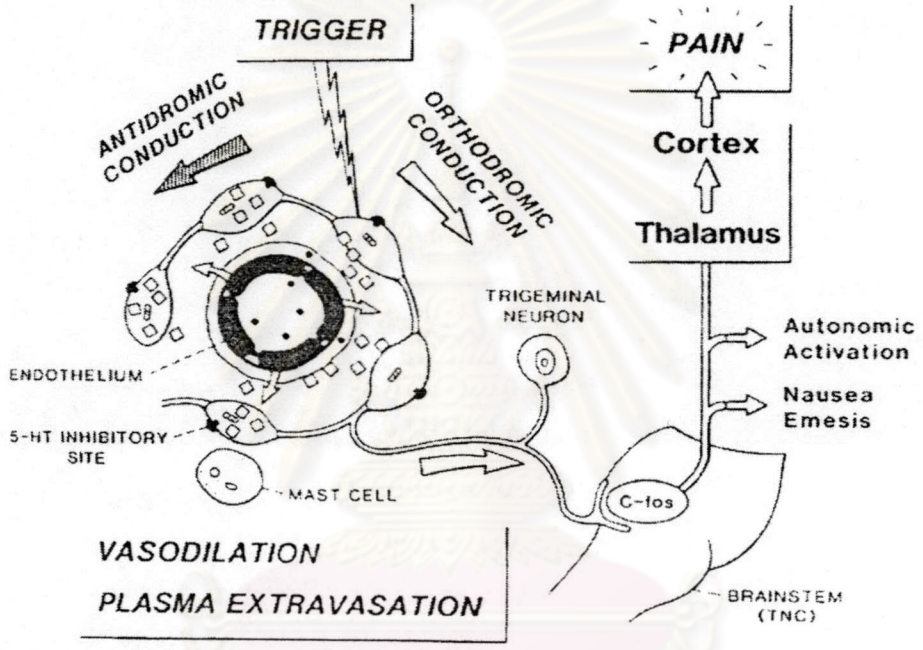

Figure 2.10 *The trigeminovascular system*

Blood vessels of the dura mater also receive rich trigeminal and upper cervical projections (Feindel et al., 1960; Mayberg et al., 1984). All trigeminal divisions innervate this important brain covering. The middle meningeal artery contains axons primarily from the ipsilateral first trigeminal division, whereas the superior sagittal sinus receives a bilateral innervation. The first and second divisions innervate the dura within the anterior fossa, and the second and third division project to the middle cranial fossa, whereas upper cervical nerves as well as vagus and trigeminal ganglia innervate dural structures within the posterior fossa.

Sensory fibers surrounding the middle cerebral and basilar arteries terminate within the trigeminal brainstem nuclear complex, including main sensory nucleus, pars oralis, and pars interpolaris (Arrab et al., 1988). Terminals are also found in the nucleus tractus solitarius, dorsal motor nucleus of the vagus, and ventral periaqueductal gray.

The neurovascular hypothesis proposes that either migraine triggers or CSD can activate trigeminal nerve axons, which then release neuropeptides (such as substance P, neurokinin A, and CGRP) from axon terminals near the meningeal and other blood vessels. Substance P and neurokinin A cause vasodilation and promote the extravasation of plasma proteins and fluid from nearby meningeal blood vessels (Markowitz et al., 1987). Although CGRP does not promote plasma extravasation, it is a potent vasodilator. Together, these neuropeptides produce an inflammatory response in the area around the innervated blood vessels. This response is termed sterile neurogenic perivascular inflammation (Moskowitz and Cutrer, 1993).

The neuropeptides may also sensitize nerve endings, providing a mechanism for sustaining the headache. When activated, the trigeminal nerve also transmits pain impulses to the trigeminal nucleus caudalis, which relays pain impulses to higher centers of the brain.

According to the neurovascular theory, vasodilation is not the cause of migraine headaches but is an accompanying phenomenon attributable to trigeminal nerve activation. Although the cause of this activation is not known, it may be due to ionic and metabolic disturbances in brain function, such as those associated with CSD. It has also been proposed that abnormal activity in brain stem sensory nuclei may cause antidromic activation of trigeminal sensory pathways.

The serotonergic abnormalities hypothesis

Observations that both plasma and platelet levels of serotonin fluctuate during a migraine attack suggest that serotonin may be involved in the pathogenesis of migraine. When platelets are activated, they aggregate and release serotonin, thus increasing the plasma serotonin level (Dalessio, 1962; Raskin, 1981). An increase in plasma serotonin level would be expected to cause vasoconstriction, whereas a decrease in serotonin would promote vasodilation.

Platelet serotonin levels may drop precipitously during the headache phase of migraine (Raskin, 1981). Also, urine levels of serotonin and its metabolites rise during headaches, suggesting that there is a large release of serotonin during such attacks (Sicuteri et al., 1961). Moreover, drugs such as reserpine that cause the release and depletion of serotonin from tissue storage sites may precipitate migraine headaches.

An initial surge in plasma serotonin levels may cause constriction of cerebral blood vessels and a reduction in cerebral blood flow. If the blood flow is sufficiently reduced, migraine aura may result. A subsequent depletion and drop in serotonin levels may then lead to a marked dilation of extracranial and intracranial arteries, precipitating migraine pain.

It seems unlikely that changes in blood serotonin levels are solely responsible for the development of migraine. For instance, global changes in plasma serotonin levels do not explain the unilateral nature of migraine pain, and serotonin levels in patients with migraine may remain depressed long after the headache has resolved. It may be, however, that changes in plasma serotonin levels reflect more important disturbances in brain serotonin levels.

The integrated hypothesis

The integrated hypothesis of migraine pathogenesis is an attempt to consolidate various theories and explain several observations related to migraine pain. According to this theory, triggers such as stress, glare, noise, the dilation of the internal or external carotid arteries or other factors may activate specific centers in the brain stem. One such center, the locus ceruleus, causes changes in epinephrine levels. Another center, the dorsal raphe nucleus, affects serotonin levels in the brain.

Constriction of cerebral blood vessels may cause a localized deficiency in blood flow, provoking CSD, which may, in turn, stimulate trigeminovascular fibers, eliciting neurogenic inflammation and headache pain. Nerve fibers from the locus ceruleus, the dorsal raphe nucleus, and the trigeminal nerve cause a stimulation of cranial nerves that dilate both cerebral and extracranial blood vessels. The dilation of meningeal vessels contributes to pain generation. The locus ceruleus also sends fibers to higher centers of the cerebral cortex, where it influences a person's state of arousal and awareness, and descending projections interact with the body's pain control mechanisms.

Likewise, the dorsal raphe nucleus sends multiple fibers to blood vessels and upward toward the cerebral cortex. These serotonin-secreting fibers help regulate sleep and neuroendocrine functions. Other connections are made with lower brain stem areas and with the hypothalamus. A disruption in the normal function of the hypothalamus may be responsible for prodromal signs and symptoms of migraine such as mood changes, food cravings, drowsiness, thirst, and yawning. These signs and symptoms may occur several hours, or even as long as 1 day, before headache pain begins.

II. NITRIC OXIDE

In 1980 Furchgott and Zawadzki reported that vasodilatation induced by acetylcholine depends on the presence of intact endothelium (Furchgott and Zawadzki, 1980). The mediator of this endothelium dependent vasodilatation was some years later identified as NO, which previously was considered to be merely an atmospheric pollutant (Palmer et al., 1987). Since then the biology of this small and short lived messenger molecule has been increasingly and very intensively investigated.

THE BIOLOGY OF NITRIC OXIDE

The highly reactive free radical NO is a lipophilic gas of formula N=O (Kiechle and Malinski, 1993). The half-life of NO is reported to be very short, in the rang of 5-30 sec under bioassay conditions (Palmer et al. 1987, Kiechle and Malinski, 1993). NO is rapidly converted to nitrogen dioxide (NO_2) which again rapidly forms the more stable metabolites nitrite (NO^-_2) and nitrate (NO^-_3) (Wennmalm and Peterson, 1991). NO is generated from the terminal guanidino nitrogen of L-arginine (Figure 2.11). The family of enzymes catalyzing NO synthesis is known as NO synthase (NOS) (Knowles and Moncada, 1994).

NOS activity has been reported in many tissues including endothelium, brain, peripheral nerves, vascular smooth muscle, myocardium, macrophages, neutrophils and microglia of several species (for review see Kiechle and Malinski 1993). Purification and cloning of NOS has revealed the existence of at least three isoforms (Table 2.2) (Knowles and Moncada 1994). Two of these are constitutive dependent, Ca^{++}/calmodulin dependent (cNOS) and release NO from endothelium (eNOS) and neurons (nNOS). This release is accelerated in response to

stimulation of several specific membrane bound receptors by e.g. glutamate, bradykinin, 5-HT, acetylcholine, histamine, endothelin-1, substance P and probably calcitonin gene-related peptide (CGRP) (Gray and Marshall 1992b, Toda 1990, Glusa and Richter 1993). Increased flow velocity and the subsequent increase of shear stress in endothelial cells may also stimulate eNOS (Figure 2.12). Another NO synthase is inducible and Ca^{2+} independent (iNOS). iNOS generates NO for long periods and in large amounts in response to endotoxins and cytokines (Figure 2.12) (Busse and Műlsch, 1990, Wallace and Bisland, 1994). Most physiological actions of NO are mediated via activation of soluble guanylate cycase (sGC) and a consequent increase in cyclic guanosine monophosphate (cGMP) eventually leading to a decrease in intracellular Ca^{2+} in target cells. (for reviews see Moncada et al., 1991; Mayer, 1994) (Figure 2.12)

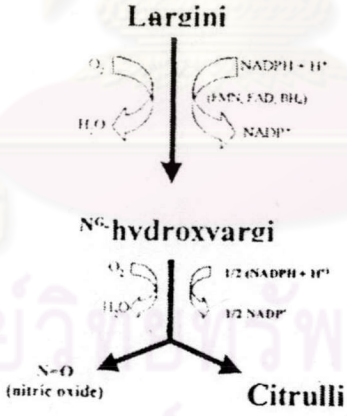

Figure 2.11 *Synthesis of NO from L-arginine. This two-step oxidation requires NADH, O_2 flavin (FMV and FAD) and tetrahydrobiopierin (BH_4) as co-factors and yields citrulline as a co-product. (From Mayer, 1994)*

Table 2.2 NOS isoforms

Type I: nNOS	Type II: iNOS	Type III: eNOS
• Activity depends on elevated Ca^{2+}	• Activity is independent of Ca^{2+}	• Activity depends on elevated Ca^{2+}
• First identified in neurons	• First identified in macrophages	• First identified in endothelial cells
• Constitutively expressed, but inducible under pathological conditions	• Inducible under pathological conditions	• Constitutively expressed, but inducible under pathological conditions
• Play a prominent role in the early stage of neuronal injury after cerebral ischemia	• Play a role in the later stage of neuronal injury after cerebral ischemia	• Play a protective role in cerebral ischemia by maintaining cerebral flow
• Protein and catalytic activity upregulated within 10 minutes and peak at 3 hours after cerebral ischemia	• Protein and catalytic activity upregulated within 12 hours and peak at 48 hours after cerebral ischemia	• Protein and catalytic activity upregulated within 1 hour and peak at 24 hours after cerebral ischemia

From Knowles and Moncada, 1994

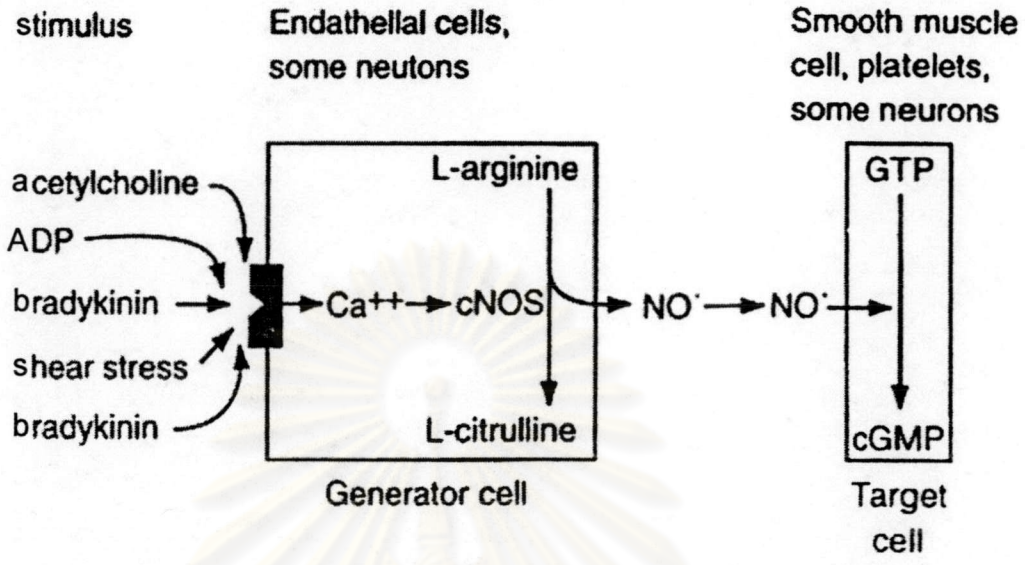

Figure 2.12 *The mechanism of NO releasing triggered by constitutive NOS.*

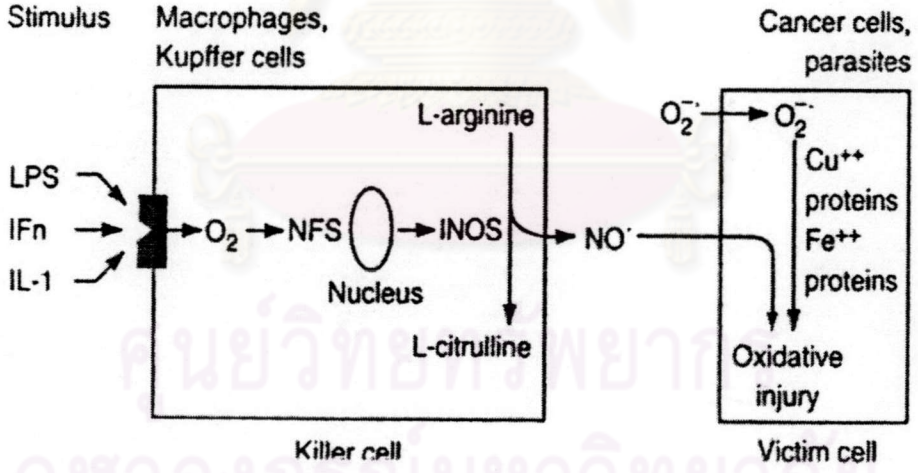

Figure 2.13 *The mechanism of NO releasing triggered by inducible NOS.*

THE ROLE OF NO IN MIGRAINE

Increasing evidence suggests that the small messenger molecule NO plays a role in migraine pain. Two different experimental human headache models-intravenous infusion of nitroglycerin (NTG) (a donor of NO) and intravenous infusion of histamine (which probably activates endothelial NO formation) offer unique possibilities in the study of NO mechanisms in migraine. The role of NO in migraine pain is reviewed mainly based on experiences from these two experimental models. Furthermore, evidence supporting the view that activation of the NO cascade of reactions is a common final pathway for headache induced by several other substances is provided. Finally physiological effects of NO relevant to migraine are discussed.

NO has an amazing number of physiologic effects throughout the body of which several, theoretically, may be implicated in the pathophysiology of migraine. Thus, endothelium-dependent vasodilatation is of importance in cerebrovascular regulation, and in addition neurogenic vasodilatation may be mediated via perivascular nerves, which operate through NO (non-cholinergic [NANC] nerves). Furthermore, NO mediates neurotransmission in the central nervous system of importance for pain perception (hyperalgesia). NO may also contribute to sensory transmission in peripheral nerves. Moreover, NO contributes to the control of platelets and, when produced in large amounts, NO contributes to host defense reactions of importance in nonspecific immunity and neurotoxicity (for review see Moncada et al., 1991). Finally, NO may release CGRP from perivascular nerve endings and may thus play a role in neurogenic inflammatory reactions (Wei et al. 1992).

NO and pain

NO has a large number of effects throughout the human body (Moncada et al., 1991). It is a free radical, which is highly reactive, and is a molecular effector of activated-macrophage-induced cytotoxicity. Furthermore, it is the most important of the endothelium derived relaxing factors (Moncada et al., 1991). After its formation, NO diffuses into vascular smooth muscle where it activates sGC. This results in the formation of cGMP which, in turn, relaxes the muscle and dilates the blood vessels (Moncada et al., 1991). NO synthesis catalyses the synthesis of NO from L-arginine, and this enzyme is present in nerve fibers surrounding cerebral blood vessels in animals and humans. However, the effect of NO formed in these nerves is unknown. NO is also found in the CNS and has been shown to play an important role in the central processing of painful stimuli (Meller and Gebhart, 1993). It facilitates the transmission of noxious impulses from the periphery to the thalamus and the neocortex and therefore enhances pain.

NO a final common pathway for several-inducing substances

Beside NTG and histamine other substances, which have been shown to reliably cause more headache than placebo in single dose experiments including reserpine, mCPP and, less convincingly, prostacyclin and hypoxia, may cause headache via NO (Pelligrino et al., 1993; Olesen et al., 1995). The best example of hypoxic headache is high altitude headache. However, no formal study of the effects of hypoxia in migraine sufferers is available. Recently, it was shown that persons living at high altitude had a huge increase in migraine prevalence (Arregui et al., 1991). Hypoxia increases longevity of NO whereas pure oxygen acts as a NO scavenger reducing the lifetime and thereby the effect of NO

(Rengasamy and Johns, 1991). Hypoxic vascular headache and hypoxia induced migraine may thus be due to increased spontaneous NO concentration. The spontaneous increased of NO was also found in migraine attacks. Recently, Stepien and Chalimoniuk (1998), measured cGMP and NO^-_2 level in the blood serum from migraine with and without aura. They found a significant increase in cGMP and NO^-_2 level in patients during migraine attack and this level decreased after the administration of sumatriptan.

Prostacyclin has been shown in one study to cause headache in migraineurs (Peatfield et al., 1981). Low concentration of exogenous PGE_2 stimulates iNOS with NO release. It is more likely that prostacyclin-induced headache via liberating NO (Meng et al., 1995).

In migraineurs, reserpine has been shown to cause headache with some migrainous features (Lance, 1991). Reserpine depletes not only platelets but also presynaptic nerve terminals of their content of monoamines. Substances released include 5-HT. The $5\text{-}HT_{2C}$ (former called $5\text{-}HT_{1C}$) receptor has recently been suggested to play a crucial role in the initiation of migraine attacks (Fozard and Kalkman, 1994). 5-HT caused an endothelium dependent relaxing response in a number of vessels from different species, and this effect was mediated via the $5\text{-}HT_{2C}$ receptor. The vascular response to $5\text{-}HT_{2C}$ activation, at least in the pig, is primarily a consequence of the release of NO (Glusa and Richter, 1993). mCPP is a direct agonist at the $5\text{-}HT_{2C}$ receptor and, therefore, is likely to cause vascular headache via NO synthesis (Fozard an Kalkrnan, 1994). Based on above data, one may be concluded that NO is a likely common denominator for headaches induced by NTG, histamine, reserpine, mCPP, prostacyclin and hypoxia.

Mechanisms of NO induced migraine

Several neurotransmitters in brain tissue, periarterial cerebral nerves and in the blood stimulate the formation of NO in brain neurons and arterial endothelium and possibly also interact with NOS containing nerve terminals (Nozaki et al., 1993; Tomimoto et al., 1994). Thus, fluctuations in neurotransmitter concentration both in brain and blood may trigger migraine headache in migraine patients due to their supersensitivity to NO. The formation of NO may be elicited by pathological reactions such as (i) CSD (Goadsby et al., 1992) (ii) the activation of the trigeminovascular system with the liberation of e.g. SP and CGRP (Fanciullacei et al., 1995 and 1997), (iii) fever and inflammation via interleukines and histamine etc. (Olesen et al., 1994).

At present it is not known in further detail how activation of the NO pathway causes migraine headache. Dilatation of large intra- and extracranial arteries may be involved because: (i) arterial dilatation is induced by NO which liberated from endothelium and probably perivascular nerve endings (Moncada et al., 1991), (ii) the cerebral vasodilatation has been reported during spontaneous migraine headache (Firberg et al., 1991; Iversen et al., 1990; Thomsen et al., 1995), (iii) mechanical dilatation of intracranial arteries causes referred pain in the areas where most patients feel their pain during migraine attacks (Nichols et al., 1990) and (iv) agents such as ergotamine and sumatriptan which constrict arteries (but not arterioles) are effective in the treatment of the acute migraine attack. On the other hand, the moderate mechanical arterial dilatation reported during migraine attacks may not be enough to cause severe pain. Another possibility is central pain modulating effects of NO. Direct activation of perivascular sensory nerve fibers and/or initiation of perivascular neurogenic inflammation (Moskowitz et al.,

1993b) by NO may be other possibilities and also the direct noxious and cytotoxic effect of NO should be considered. Whatever is true, it is striking that NO cause migraine with a delay of up to several hours (Thomsen et al., 1994a,b). A time course that mimics the often slowly progressing development of migraine pain during spontaneous migraine attacks. As mentioned, NO is an unstable free radical with a very short half-life. Other mediators or mechanisms therefore seem to be involved in the rather slow cascade of events set up by activation of the NO pathway and eventually leading to a migraine attack. The elucidation of these mechanisms and of steps further down the NO activated cascade of reactions is a fascinating future challenge likely to provide new therapeutic approaches to migraine.

NOS inhibition may be a new therapeutic principle in migraine

NOS inhibition is a novel principle in the treatment of migraine. 546C88 was the only NOS inhibitor available for clinical use which conformed to the regulations of the Danish National Health authorities. 546C88, a non-selective NOS inhibitor, inhibits all three types of NOS. An intravenous infusion of 546C88, a NOS inhibitor, may be effective in the acute treatment of migraine attacks. Two hours after the infusion, 10 to 15 (67%) 546C88-treated patients experienced headache relief compared with 2 of 14 (14%) placebo-treated patients ($p<0.05$). Symptoms such as phono- and photophobia were also significantly improved. There was no significant difference between the 546C88-treated patients and the placebo-treated patients in the relief of nausea. Therefore, they conclude that the effect of 546C88 on migraine is likely to be a specific result of NOS inhibition and decreased NO formation rather than an effect of cerebral vasoconstriction (Lassen et al., 1998).

According to previous studies, NO may initiate migraine attacks, so NOS inhibitors should also be evaluated for their effect as prophylactic agents in migraine. For this purpose selective inhibitors without systemic circulatory effects would clearly be needed.

III. SEROTONIN (5-HT)

Serotonin (5-HT), a biogenic amine with wide distribution in both the plant and animal kingdoms, is the vasoconstrictor substance in serum which was identified, crystallized and named by Rapport et al in 1948. While independently characterizing the substance that gives the enterochromaffin cells of the gastrointestinal mucosa their unique histochemical property, Erspamer (1956) found that this compound was 5-hydroxytryptamine and was identical to serotonin. The structure of 5-HT is shown in figure 2.14. The combination of the hydroxyl group in the 5 position of the indole nucleus and a primary amine nitrogen serving as a proton acceptor at physiological pH makes 5-HT a hydrophilic substance. As such, it does not pass the lipophilic blood brain barrier readily. Thus, its discovery in brain indicated that 5-HT was being synthesized in brain, where it might play an important role in brain function. In man, 90% of the 5-HT is found in the enterochromaffin cells of the gastrointestinal mucosa. The remainder is found in platelets and the central nervous system (CNS) (Sjoerdsma et al., 1970)

The serotonergic neuronal system is uniquely organized with cells of origin in the brainstem which provide extensive projections to virtually all areas of the brain and spinal cord. In addition, there are serotonergic neurons that originate from the midbrain raphe and innervate cerebral blood vessels. When activated, these neurons change cerebral blood flow (Lance, 1992). Specific 5-HT receptor subtypes are localized to the vascular structures innervated by serotonergic neurons (Lance, 1992).

Apart from its role as neurotransmitter in the CNS, 5-HT appears to act as a modulator, altering the level of sensory responsiveness or motor activity but not actually mediating the responses (Boadle-Biber,

1993). 5-HT has been implicated in controlling feeding behavior, thermoregulation, sexual behavior, sleep, and pain modulation (Leonard, 1992). The development of selective serotonergic receptor agonists and antagonists has revolutionized the treatment of headache.

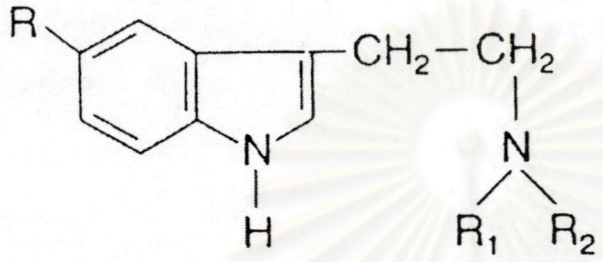

Figure 2.14 *Chemical structure of 5-hydroxytryptamine (5-HT)*

5-HT SYNTHESIS AND METABOLISM

Neurons and enterochromaffin cells synthesize 5-HT from the amino acid, L-tryptophan, while platelets acquire it from the blood (Sjoerdsma et al., 1970). The biosynthesis and catabolism of 5-HT are shown in figure 2.15. The first step in biosynthesis is catalyzed by the enzyme tryptophan 5-hydroxylase (the rate-limiting enzyme), which converts L-tryptophan to 5-hydroxytryptophan (5-HTP). 5-HTP is decarboxylated to 5-HT by the nonspecific aromatic L-amino acid decarboxylase. In neurons, 5-HT is taken up into secretory granules and stored. In man, 5-HT is mainly oxidatively deaminated by monamine oxidase (MAO) to form 5-hydroxyindoleactetaldehyde. The aldehyde is rapidly degraded by aldehyde dehydrogenase to 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of 5-HT.

5-HT synthesis is regulated by modulating the rate of conversion of L-tryptophan to 5-HTP .The concentration of tryptophan is subsaturating for tryptophan hydroxylase. Administration of exogenous tryptophan leads to a rise in brain levels of tryptophan and an increase in 5-HT synthesis in rats (Boadle-Biber, 1993). This effect depends on the rate of firing of the 5-HT neuron and does not occur if firing rates are reduced. Electrical stimulation enhances 5-HT production by increasing tryptophan hydroxylase activity, most likely by enzyme phosphorylation. Activation of the somatodendritic 5-HT_{1A} autoreceptors inhibits neuronal firing and 5-HT synthesis. Activation of the terminal autoreceptor inhibits the synthesis and release of 5-HT in the absence of any effect on firing rate (Boadle-Biber, 1993).

5-HT exists in several pools, and newly synthesized 5-HT is preferentially released from the storage vesicles in response to neuronal stimulation. The action of 5-HT is mainly terminated by reuptake into the nerve terminal by the 5-HT-transporter (Boadle-Biber, 1993). 5-HT interacts with its target sites through various receptors, some of which are modulated by estrogens; most migraine drugs are believed to interact with these receptors. Many receptors have been cloned and their amino acid sequence and tertiary structure established (Peroutka, 1993).

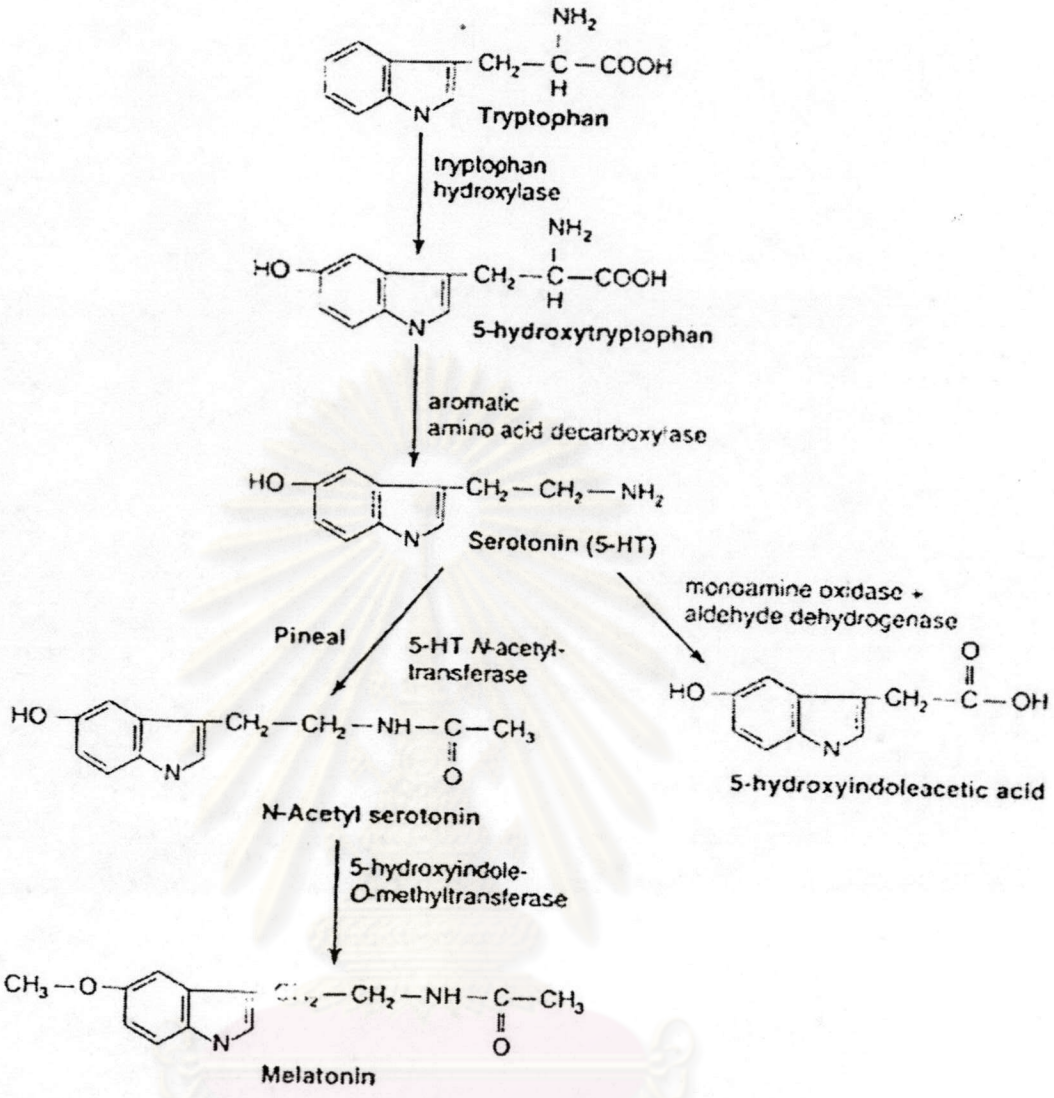

Figure 2.15 *The biosynthesis and catabolism of serotonin*

5-HT RECEPTORS

5-HT recognizes at least three distinct types of molecular structures: guanine nucleotide binding G protein-coupled receptors, ligand-gated ion channels, and transporters (Table 2.3) (Silberstein, 1994). Prior to the introduction of molecular biological techniques, the classification of 5-HT receptors was based mainly on their pharmacologic properties. The existence of 5-HT receptor subtypes was first demonstrated by Gaddum and Picarelli (1957), who examined 5-HT-induced contractions in the guinea pig ileum. Using pharmacologic antagonists, they postulated the existence of a D 5-HT receptor on smooth muscle and an M 5-HT receptor on the parasympathetic ganglia (Table 2.4). Radioligand binding techniques have led to the identification of increasing numbers of receptor subtypes. Peroutka and Snyder (1979) demonstrated two distinct subtypes in the CNS: the 5-HT_1 and the 5-HT_2, based on their affinity for radioactive [$_3$H]-5-HT. The 5-HT_2 corresponded to the D, while the 5-HT_1 was a new receptor type. Bradley et al (1986) later incorporated both nomenclatures: 5-HT_1 became 5-HT_1-like and were defined by their susceptibility to antagonism to methiothepin, resistance to 5-HT_2 antagonists and potent agonist by 5-carboxamidotryptamine (5-CT); D continued as 5-HT_2, and M became 5-HT_3. Molecular biological data have confirmed the existence of at least 7 different families of receptors (Table 2.5) The Serotonin Club has recently proposed a new nomenclature for 5-HT receptors based on their operational, structural and transduction properties (Table 2.5) (Humphrey et al., 1993).

Table 2.3 Overview of 5-HT Recognition sites

G protein coupled receptors
5-HT_1
5-HT_2
5-HT_3
5-HT_4
5-HT_5
5-HT_6
5-HT_7
Ligand gated ion channels
5-HT_3
Transporters
5-HT uptake site

Table 2.4 5-HT Receptor Nomenclature

1957							
Gaddum	D	M					
Picarelli							
1979							
Peroutka&	$5HT_1$	5-HT_2					
Snyder							
1986	"$5\text{-HT}_{1\text{-like}}$"	5-HT_2	5-HT_3				
Bradley et al.							
1993	$5HT_{1A}$	$5HT_{2A}$	$5HT_3$	$5HT_4$	$5HT_{5A}$	$5HT_6$	$5HT_7$
Serotonin	$5HT_{1B}$	$5HT_{2B}$			$5HT_{5B}$		
Club $5HT_{1D}$	$5HT_{2C}$						
	$5HT_{1E}$						
	$5HT_{1F}$						
Transducer	↓cAMP	↑P_1	ion channel	↑cAMP?	↑cAMP	↑cAMP	

Table 2.5 Classification of Serotonin Receptors

5-HT_1
- G protein linked
- Inhibits adenylate cyclase
  - 5-HT_{1A}
  - 5-HT_{1B}
  - 5-HT_{1D}
  - 5-HT_{1E}
  - 5-HT_{1F}

5-HT_2
- G protein linked
- Prosphatidylhydrolysis
  - 5-HT_{2A}
  - 5-HT_{2B}
  - 5-HT_{2C}

5-HT_3
- linked to ion channel

5-HT_4
- G protein linked
- Stimulates adenylate cyclase

5-HT_5
- G protein linked
  - 5-HT_{5A}
  - 5-HT_{5B}

5-HT_6
- G protein linked
- Stimulates adenylate cyclase

5-HT_7
- G protein linked
- Stimulates adenylate cyclase

The 5-HT_1 family includes subtypes which can be grouped together based on the absence of introns in the cloned genes, a common G-protein transduction system (inhibition of adenylate cyclase), and similar operational characteristics. Potent agonist by 5-CT is no longer required to define 5-HT I receptors.

The 5-HT_1 family of inhibitory receptors includes subtypes A, B, D, E and F. The 5-HT_1 G protein linked receptors inhibit the production of cyclic adenosine monophosphate (cAMP), while the 5-HT_2 G protein linked receptors stimulate phosphoinositol hydrolysis. Other G protein-coupled receptors are the 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptors. The 5-HT_3 receptor is coupled to an ion channel (Saudou and Hen, 1994).

The 5-HT_{1A} receptor, the first cloned human 5-HT receptor, has a high selective affinity for 8-hydroxy-2- (di-n-propylamino)-tetralin (8-OH-DPAT). Activated human 5-HT_{1A} receptors expressed in HeLa cells inhibit forskolin-stimulated adenylate cyclase activity. Drugs such as buspirone act as agonists in cell lines with a large number of receptors and as antagonists in cell lines with few receptors, suggesting that this property of a ligand is dependent on receptor density.

It was formerly believed that there was a separate 5-HT_{1D} in humans and 5-HT_{1B} in rodents, but it has been shown that the B receptor exists in humans and the D receptor in rodents. The rodent 5-HT_{1B} receptor is 97% homologous to the human receptor. This has been alternatively named the 5-$HT_{1D\beta}$ receptor while the classic 5-HT_{1D} receptor is called 5-$HT_{1D\alpha}$. Despite the homology, the receptors have different pharmacologic profiles. Several β-adrenoceptor antagonists have high affinity for the rodent but not the human 5-HT_{1B} receptor (Maroteaux et al., 1992).

5-HT_{1C}, originally named a "1" receptor based on its high affinity for 5-HT, behaves like a 5-HT_2 receptor based on second messenger and other properties and has tentatively been renamed 5-HT_{2C} (Humphrey et al., 1993).

The 5-HT_{1D} receptor was originally identified in bovine brain membrane by Heuring and Peroutka (1987). The human receptor has been cloned and first reported by Hamblin and Metcalf (1991). No selective agonists exist. The 5-HT_{1D} receptors are the most common 5-HT receptor subtype in the human brain and may be identical to the 5-HT_1-like receptor in the cranial vasculature. Sumatriptan and the ergot alkaloids have high affinity for both the human 5-HT_{1D} and 5-HT_{1B} receptors (Heuring and Peroutka, 1987).

The cloned human 5-HT_{1E} receptor has low affinity for 5-CT and sumatriptan, unlike other 5-HT_1 receptors (Zgombick et al., 1992)

The cloned human 5-HT_{1F} receptor, like its closest genetic relative, the 5-HT_{1E} receptor, has low affinity for 5-CT but differs in its high affinity for sumatriptan (Adham et al., 1993).

The 5-HT_2 receptors have close sequence homology, similar intron/exon gene products, the same G-protein linked transduction system (stimulation of phospholipase C) and similar operational profiles. The Serotonin Club has renamed the 5-HT_2 classic receptor, 5-HT_{2A}. The 5-HT_{2B} receptor is the 5-HT_{2F} fundus receptor. It has been proposed that the 5-HT_{1C} receptor be renamed the 5-HT_{2C} receptor as it behaves like a 5-HT_2 receptor (Baxter et al., 1995).

The 5-HT_3 receptors are distinct, as they belong to the ligand-gated ion channel receptor superfamily. The selective antagonists ondansetron and granisetron are antiemetics.

The 5-HT_4 receptors are G protein coupled receptors positively linked to adenylate cyclase.

The 5-HT_5 family, cloned from the mouse genome library, is expressed predominantly in the CNS. Two distinct varieties have been found: 5-HT_{5A} and 5-HT_{5B}. Gene coding is interrupted by one intron. The 5-HT_{5A} and 5-HT_{5B} receptors, while similar, are found on different chromosomes. The 5-HT_5 receptors have a high affinity for ergot alkaloids and 5-CT, a low affinity for 5-HT, and are G protein linked.

The 5-HT_6 receptor has been cloned in rats. It is positively linked by a G protein to adenylate cyclase and is exclusively localized to the CNS. When expressed in mammalian cells, it has high affinity for 5-HT and ergot alkaloids. Some antidepressants and antipsychotics show high binding affinity.

The 5-HT_7 receptor has been cloned in rats. It is positively linked by a G protein to adenylate cyclase. Ergot alkaloids, methiothepin, 5-HT, 5-CT, 8-OH-DPAT, and some antidepressants and antipsychotics show high, binding affinity. Both methiothepin and clozapine inhibit 5-HT stimulation of cyclic AMP by this receptor when it is expressed in mammalian cells (Shen et al., 1993).

THE ROLE OF 5-HT IN MIGRAINE

5-HT is clearly involved in migraine; the drugs that treat migraine symptoms most effectively are known to interact with serotonin receptors. But the nature of that involvement is unclear. Since there is little evidence as yet to show that serotonin disturbances actually cause migraine, many researchers are focusing on the practical aspects of the connection, working on developing new therapies that target specific serotonin receptors. This article presents an overview of the evolving knowledge linking serotonin and migraine.

5-HT is a naturally occurring chemical that is widely distributed in the body. Large concentrations of 5-HT are found in the gastrointestinal tract (90%), the platelets (8%), and the brain (Saper et al., 1993).

The first hint that 5-HT might be important in migraine came nearly 40 years ago, with the discovery that the drug methysergide antagonized certain actions of 5-HT and could be used to prevent or reduce the intensity and frequency of migraine attacks (Raskin, 1991).

Subsequent studies revealed other possible links between migraine and serotonin. It seems that levels of serotonin in the blood fall at the onset of headache but are normal between attacks. Urinary excretion of the serotonin metabolite (5-HIAA) acid also increases during an attack and returns to normal afterwards. Researchers also observed that migraine can be precipitated by the drug reserpine, which depletes serotonin, and relieved by serotonin agonists, meaning the agonists combine with the receptors and stimulate serotonin activity.

These findings led to a search for safe and effective antimigraine agents that could interact with serotonin receptors in the brain (Goadsby,

1997). Drugs that blocked or enhanced serotonin release from nerve cells, the thinking went might somehow influence the frequency and/or severity of migraine attacks.

Serotonin receptors, agonists, and antagonists

When the search for antimigraine agents that selectively target serotonin began in the early 1970s, two 5-HT receptors already had been discovered. Further investigation resulted in the identification of 5 additional receptors (5-HT_3 - 5-HT_7) , as well as several subtypes of each. The 5-HT_1, 5-HT_2, and 5-HT_3 receptors are most relevant to migraine.

5-HT_1 receptors

The 5-HT_1 receptors are widely distributed in the brain, particularly in intracranial blood vessels and in the dorsal raphe nucleus and nucleus raphe magnus in the brain stem, which some experts believe is the site of the "migraine generator" (Weiller et al., 1995).

The 5-HT_1 receptors especially the 5-HT_{1D} subtype are among the targets of currently available drugs that treat acute migraine attacks, namely sumatriptan, dihydroergotamine (which also acts on 5-HT_{1A} receptors), and ergotamine (Rapoport and Sheftell, 1996). These collectively are called the serotonin agonists.

Because serotonin constricts blood vessels, many experts believe that serotonin agonists relieve migraine headache by constricting swollen cranial blood vessels, thereby reducing inflammation around the vessels and reducing pain transmission through the trigeminal system (Mathew, 1997). However, there is no consensus on this point among migraine specialists. There are other data suggesting that the blood-brain barrier "opens" during the migraine and may allow sumaptriptan or

dihydroergotamine to enter the brain and reach neurons, possibly even those in the proposed "migraine center".

A "new generation" of triptan drugs, believed to target serotonin even more specifically than does sumatriptan, will be available in the near future. They include zolmitriptan, rizatriptan, naratriptan, eletriptan, and alniditan. Studies have indicated that these serotonin agonists may have a quicker onset and longer duration of action than sumatriptan, as well as fewer side effects. Head-to-head comparisons, however, have been done in only a few cases.

5-HT_2 receptors

Drugs that block the actions of serotonin by acting on 5-HT_2 receptors (serotonin antagonists) can be useful in preventing migraine. These include methysergide, the tricyclic antidepressant amitriptyline (Tfelt-Hansen, 1997), and certain beta blockers and calcium channel blockers.

5-HT_3 receptors

There is some evidence that the gastrointestinal symptoms associated with migraine, such as nausea and vomiting, may involve 5-HT_3 receptors, which are found in the medulla site of the "vomiting centers" in the brain. Some experts speculate that these centers are activated during a migraine attack and that 5-HT_3 antagonists may be useful in preventing migraine-associated nausea and vomiting. The anti-emetic drug odansetron is a 5-HT_3 antagonist.

Serotonin and migraine-related disorders

Studies suggest that people with migraine are predisposed to such mood disorders as anxiety, panic attacks, and depression; some experts believe these mood disturbances may actually be part of a "migraine syndrome". Interestingly, fluctuations in serotonin have been implicated as a cause or trigger of these mood disorders, further strengthening the link between serotonin and migraine.

Once more is known about the mechanisms of head pain, other migraine symptoms and associated mood disorders, the role of serotonin and its various receptor types in migraine will also be clarified. Knowledge accumulated during the past few decades has led to the development of new antimigraine drugs that are effective, at least in part, because of their ability to interact with serotonin receptors. Ongoing and future investigations into the serotonin-migraine connection will likely produce even more effective therapies for both acute treatment and prevention.

The aims of this study are:-

1. To study the effect of CSD on interaction between circulating leukocytes and pial endothelium.

2. To study the effect of CSD on rCBF and pial arteriolar diameter.

3. To study the effect of NOS inhibitor on rCBF and MABP.

4. To study the effect of NOS inhibitor on CSD-evoked cerebral hyperemia.

5. To study the effect of 5-HT_{1B} receptor agonist on rCBF and MABP.

6. To study the effect of 5-HT_{1B} receptor agonist on CSD-induced cerebral hyperemia.