CHAPTER II
REVIEW LITERATURE

REVIEW OF MIGRAINE

The word 'migraine' is of French origin and derives from the Greek 'hemicrania' like the old English term 'megrim'. Although hemicrania literally means only half of the head, migraine involves both sides of the head from its onset in about 40 per cent of patients. Another 40 per cent experience strictly unilateral headaches and approximately 20 per cent start on one side and later become generalized (Selby and Lance, 1960).

DEFINITION AND CLASSIFICATION OF MIGRAINE

Migraine is essentially an episodic headache, usually accompanied by a large number of associated symptoms such as nausea and/or photophobia. There may be great diversity in expression in terms of location, type, and amount of pain and a variable number of symptoms other than pain. Moreover, the symptoms of migraine vary from one patient to another and even between recurrent attacks in the same patient. For this reason, a good definition of the migraine syndrome has been long in coming. In 1988, the International Headache Society (IHS) proposed the following diagnostic criteria for migraine headache that the patient must have had five or more attacks of headache, lasting usually from 4 to 72 hours if untreated, with at least two of four features (unilateral, pulsating quality, moderate or severe intensity and aggravated by activity) as well as often be associated with nausea, with or without vomiting or photophobia and phonophobia. (Table 2.1)

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 manifestations 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

Source: Headache Classification Committee, 1988.

Based on this classification, migraine can be clarified into two main subgroups: 1) migraine with aura and 2) migraine without aura. Migraine with aura is used to describe migraine headache occurring in association with neurological or visual symptoms. Migraine without aura is used to describe migraine without neurological or visual symptoms. Table 2.1 summarizes the classification of migraine and the criteria for making these diagnoses.

PATHOPHYSIOLOGY OF MIGRAINE

The exact pathogenesis of migraine is not known, but it is thought to be regard as a hereditary sensitivity of neurovascular reactions to certain stimuli or to cyclic changes in the central nervous system. The pathophysiological basis of migraine remains unknown. However, several theories have been proposed to explain this common medical problem. Some of the major hypotheses regarding migraine pathogenesis are itemized below.

1. The Vascular Theory

Abnormalities of cerebral blood flow appear to play a pivotal role in the pathogenesis of migraine. The pulsating quality of headache indicates vascular connection. However, it is still debated whether vasoconstriction or vasodilatation can account for migraine headache.

Vasoconstriction: Based on the theory of Wolff, developed in the 1940s and 1950s, migraine was considered to be a vasospastic disorder; cerebral vasoconstriction was postulated to occur during the migraine prodrome and vasodilatation to occur during the headache phase (Wolff, 1987). In apparent support of this theory, a number of studies have

documented a decrease in cerebral blood flow during the aura and increase during the headache phase of migraine (Lance, 1981).

Vasodilatation: One of the mechanisms of pain production in migraine is the dilatation of arteries lying outside the brain, especially scalp arteries. There is an alleged correlation between the severity of headache and the pulse amplitude of scalp arteries (Tunis and Wolff, 1953). Artificial distention of the superficial temporal artery can reproduce a migraine headache; physical compression or chemical constriction of scalp arteries often alleviates headache at least temporarily. Two-third of migraineurs experience relief if the carotid artery is occluded ipsilateral to the side of headache, although this does not account for the remaining one-third at all (Drummond and Lance, 1983). Moreover, distention of major cerebral vessels by balloon dilatation leads to pain referred to the opthalmic division of the trigeminal nerve (Nichols et al., 1993). The direct measurement confirms that extracranial blood flow is often elevated during migraine attacks (Sakai and Meyer, 1978). Moskowitz et al. (1989) has shown that SP, a peptide neurotransmitter that dilates pial arteries is released by trigeminal nerve fibers into the walls of cerebral vessels, increases vascular permeability, and activates cells that participate in the inflammatory response can produce migraine pain.

A recent study using combined transcranial sonography and rCBF technique, shown that unilateral headache is associated with dilatation of large intracranial arteries on the headache side (Friberg et al., 1991) (Figure 2.1). It is well known that the large intracranial arteries contain nociceptive fibers derived from the trigeminal ganglion. So, It is likely that migraine headache is associated with the dilatation of the large intracranial arteries.

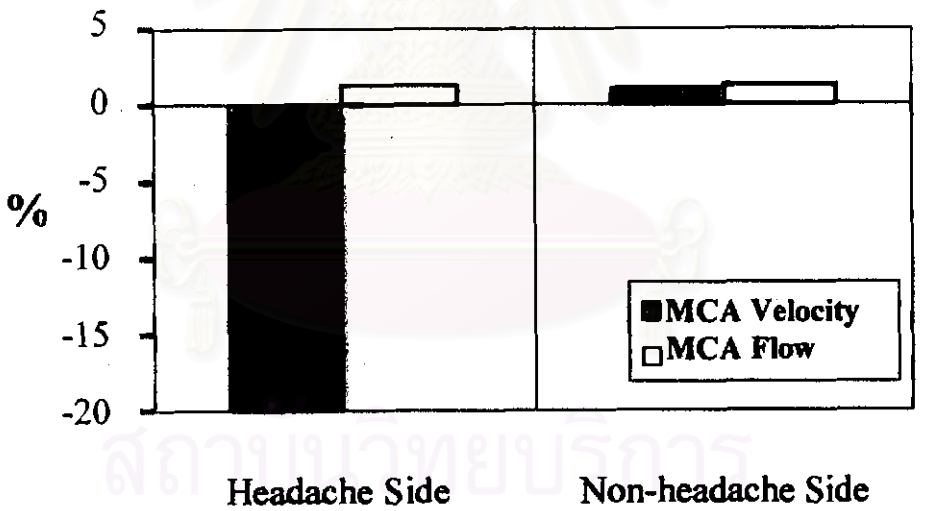

Figure 2.1. Middle cerebral artery, MCA, velocity recorded with transcranial ultrasonography was significantly reduced on the headache side but normal on the non-headache side. rCBF was normal on both sides. The data indicated a 20% dilatation of the MCA on the headache side, which was normalized after treatment with sumatriptan causing headache recovery. Data from patients *with* and *without* aura modified from Friberg et al., 1991.

Many evidence show that cerebral vasodilatation response to carbon dioxide is greater in migraine patients than in normal controls (Olesen et al., 1993; Thomsen et al., 1993b). Moreover, the reaction of extracranial arteries to exercise and stress (Drummond and Lance, 1981) is greater on the side of their usual migraine headache. These evidence show that the cerebral blood vessels from migraineurs may be susceptible for noxious stimuli than non-migraineurs.

Headache following the administration of vasodilators, such as nitrates, has been used to support the importance of a vasodilatation cause. Wei and coworkers (1992) have determined that the mechanism of dilatation by NTG and sodium nitroprusside in the cat is via the activation of perivascular sensory nerves, with subsequent release of CGRP. This in turn relaxes vascular smooth muscle via activation of the soluble form of guanylate cyclase. This would cause the vessels to change diameter and trigger the nociceptive perivascular fiber. It is highly likely that it is not the vasodilatation per se that causes headache in response to NTG, but the activation of trigeminal perivascular sensory afferent nerves fibers by NO may participate.

In conclusion, the ability of these changes in cerebral blood flow (vasoconstriction/vasodilatation) to induce the symptoms of migraine has been questioned. The observed decrease in blood flow does not appear to be significant enough to cause focal neurological symptoms. *Secondly,* the increase in blood flow per se is not painful, and vasodilatation alone cannot account for the local edema and focal tenderness often observed in migraine patients. Thus, it is unlikely that simple vasoconstriction and vasodilatation are the basic pathophysiological abnormalities in migraine.

However, it is clear that cerebral blood flow is altered during certain migraine attacks.

2. The Cortical Spreading Depression

Cortical spreading depression (CSD) is a disorder that can be accompanied by both increases and decreases of perfusion (Olsen et al., 1987). It is a transient disturbance of mechanisms maintaining ionic homeostasis of the brain and is suggested to account for the neurological symptoms associated with migraine with aura and to participate with migraine attacks. This phenomenon was first characterized and detected by Leao, as an one or repeated waves of neuronal discharge that slowly moves along the brain cortex surface in response to noxious stimuli. A focal reduction in electrical activity and increase in blood flow occur and then spread across the hemisphere at the rate of 2 to 3 millimeters per minute (Leao, 1944). During CSD, depolarization of neurons and glial cells occurring, giving rise to spike activity followed by neuronal silence. The sequence of brief excitation followed by a short-lasting depression is believed to be the basis of sensory symptoms during migraine aura (Lauritzen, 1994). 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 (Shibata et al., 1994; Wahl et al., 1994).

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. Importantly, these blood flow changes did not correspond to the distribution of the major intracranial arteries. However, the observed flow changes were similar to the electrical phenomenon of CSD of Leao. Olesen et al., (1982) speculated that the aura of classic migraine may be occur secondary to the spreading oligemia observed in classic migraine patients. This theory states that migraine results from an evolving process in the cerebral cortex that occurs secondarily to decreased 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 oligemia 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, regional cerebral blood flows 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.

Apart from the blood flow changes, CSD is associated with marked disturbances of extracellular and intracellular ion concentrations. For example, the 10-fold increases of $[K^+]_o$ and 10-fold decreases of $[Ca^{2+}]_o$ (Gardner-Medwin, 1996). These perturbations in ionic homeostasis may be caused by a negative shift of extracellular direct circuit potential, leading to a cell depolarization. The precise mechanisms involving the spread of activity are thought to involve activation of N-methyl-D-aspartate (NMDA) receptors, as propagation and induction of spreading depression are both blocked by NMDA receptor blockers such as MK801 and DL-2-aminophonovaleric acid (Parsons et al., 1996).

While the theory of CSD is interesting, there is no convincing evidence that CSD can be certainly elicited in the human brain, since this electrical phenomenon has never been recorded in human beings during a migraine attack.

3. Neurogenic Inflammation

Neurogenic inflammation (manifested as vasodilatation and plasma protein extravasation) has long been implicated in the pathogenesis of headache. Lewis (1937) proposed the term *nocifensor* system to identify the role of neurogenic inflammation as a potent endogenous defense in the early phase of tissue damage. As in other tissue, neurogenic inflammation in the dura involves the release of the vasodilator neuropeptide, CGRP, SP (Lembeck, 1953) and neurokinin A (NKA) (Saria et al., 1985). SP is the most important of these neuropeptides. Antagonists to the SP receptor [NK_1 antagonists] potently block neurogenic inflammation within the meninges (Connor and Beattie, 1996). Neurotransmitter release elicited by electrical trigeminal

stimulation results in vasodilatation, platelet aggregation, mast cell degranulation, and an increase in vascular permeability within ipsilateral dura mater (Markowitz et al., 1987). These response can be abolished by neonatal capsaicin pretreatment which destroy unmyelinated sensory afferent neurons (Markowitz et al., 1987; Dimitriadov et al., 1991). Systemic administration of the tachykinins mimics some of these changes. Leakage occurs primarily in postcapillary venules, as demonstrated by horseradish peroxidase (HRP) light and electron microscopy studies (Dimitriadov et al., 1992). Moskowitz and Cutrer have provided an elegant series of experiments whose results suggest that the pain of migraine may be a form of sterile neurogenic inflammation (Moskowitz and Cutrer, 1993). Neurogenic plasma extravasation can be seen during electrical stimulation of the trigeminal ganglion in the rat (Markowitz et al., 1987). Plasma extravasation can be blocked by ergot alkaloids (Markowitz et al., 1988), indomethacin, acetylsalicylic acid (Buzzi et al., 1989), 5-$HT_{1B/D}$-like agonist, sumatriptan (Buzzi and Moskowitz, 1990), gamma-aminobutyric acid (GABA) agonists such as valproate and benzodiazepines (Cutrer et al., 1995a), neurosteroids (Limmroth et al., 1996), SP antagonists (Cutrer et al., 1995b), and endothelin antagonist bosentan (Brandli et al., 1996; Kallela et al., 1998). Recently, the role of neurogenic inflammation in the pathogenesis of migraine has been questioned. It seems that blocking neurogenic inflammation without vasoconstriction is not enough to abort a migraine attack: bosentan, a blocker of neurogenic inflammation (in a rat model) with no vasoconstrictive activity, is not effective in migraine (May et al., 1996).

4. Serotonergic Abnormalities

Serotonin is a biogenic amine neurotransmitter that has been implicated in the pathogenesis of migraine. It is likely that an imbalance of the serotonergic transmitter and receptor system of the vessels is involved in the pathophysiological mechanisms of migraine. 5-HT itself causes constriction of the large arteries and dilatation of the resistance vessels. Biochemical studies have documented abnormalities of serotonergic systems in migraine (Dalessio, 1962; Raskin, 1981). For example, plasma and platelet levels of 5-HT have been reported to vary during different phases of the migraine attack (Raskin, 1981). At the same time, increased amounts of 5-HT and its metabolite, 5-HIAA, are excreted in the urine during most headache attacks and circulating 5-HT levels were found to fall during attacks (Sicuteri et al., 1961).

5-HT-like substance could counteract the cerebrovascular alterations seen during a migraine attack. Intravenous injection of 5-HT agonist tends to reverse migraine headache (Kimball et al. 1985). Hopf et al., (1992) described a patient with migraine with aura whose headaches subsided with the development of a 5-HT-secreting carcinoid tumour and returned after its surgical removal. Friberg et al., (1991) showed that the middle cerebral artery dilatation detected during migraine attack can be reversed by sumatriptan, a 5-HT_{1D} agonist. (For review; see review of serotonin and headache).

5. Genetic

It is very likely, but still not proven that hereditary factors play a role in the individual susceptibility to develop migraine attack. Significant data have existed for many years to indicate that migraine is a genetically

transmitted syndrome. Indeed, Liveing (1873) noted the frequent occurrence of "megrim" within families and that the disorder was often transmitted from parent to child. The study of twin pairs by Lucas in 1977 can also provided very important information about the contribution of hereditary factors to a migraine headache. This interesting finding was that monozygotic twins, who were separated at birth and raised apart not only were concordant for the occurrence of migraine, but also for the age at onset of the attacks. This observation in twins strongly supports a genetic component of migraine. Moreover, the hypothesis that migraine is inherited has been supported by numerous studies (Allan, 1928; Russell et al., 1993). Some authors have even considered a positive family history as a prerequisite for the diagnosis of migraine (Sjaastad and Stovner, 1993). Traditionally, family studies have been used to determine whether a migraine headache has a genetic basis (Ferrari et al., 1996)

Prior to recent technical advances in the field of molecular genetics, it was essentially impossible to even consider migraine as a candidate disease for a formal genetic analysis. The wide spectrum of clinical symptomatology, the likely role of environmental factors such as stress and diet and perhaps most important, the high prevalence of migraine within the general population are all factors which make the genetic analysis of migraine a difficult task. However, the development of the polymerase chain reaction technique has led to an exponential growth in the ability to generate data on the human genome. Since, the molecular genetic analysis of migraine can be performed. The report by Joutel and colleagues in 1993 showed the genetically linking a region of DNA on human chromosome 19 to the clinical diagnosis of familial hemiplegic migraine (Joutel et al., 1993). May and co-workers (1995) provided evidence for the involvement of a gene on chromosome 19 in the etiology

of common forms of migraine. They suggested that the putative 19p13 gene play a role in a number of migraine families. Recently, Ophoff et al., (1997) identified some mutations of brain-specific P/Q-type calcium-channel α_1 subunit gene on chromosome 19p13.1 and suggested that these mutations may play role in migraine pathogenesis. However, it remains to be elucidated whether this genetic contribution constitutes a mild risk factor for migraine in the general population.

6. Magnesium deficiency

Glutamate-induced CSD can be blocked by magnesium ion (Mg^{2+}) (Van Harreveld, 1984). CSD can also be more easily evoked in brain cortex deficient in Mg^{2+} (Mody et al., 1987). The measurement of brain phosphates by nuclear magnetic resonance imaging after the intravenous injection of ^{31}P has enabled the indirect assay of Mg^{2+} content by examining the chemical shift properties of the ^{31}P resonance signals. By using this non-invasive technique, Welch et al., (1989) found that intracellular Mg^{2+} concentration appear to be reduced in the brain of migraine patients during an attack. Because of the cross-sectional nature of this study, no patients were studied both during an attack and between an attack. As a result, it was impossible to ascertain whether the lower Mg^{2+} was the result of the migraine attack, was established before the attack or was a constant feature of the migraine brain interictally.

Few studies have attempted to establish a relationship between migraine and Mg^{2+}. Migraine sufferers excrete Mg^{2+} in increased amounts as a result of stress leading to transient hypomagnesemia in the serum (Durlach, 1976). Jain et al., (1985) found lower CSF Mg^{2+} in

migraine patients when compared with controls. Schoenen et al., (1991) has reported low red blood cell Mg^{2+}.

Mg^{2+} normally gates the NMDA receptor (Nowak et al., 1984) and low extracellular Mg^{2+} might render the receptor increasingly sensitive to CSD. This suggests a basis for cerebral hyperexcitability, which could make the brain susceptible to CSD and increase downstream drive onto hypothalamic and brainstem nuclei.

7. Excitatory amino acids

Glutamic and aspartic acid are excitatory neurotransmitters of the CNS. Excessive ingestion of glutamate may trigger migraine symptoms in predisposed patients (Reif-Lehrer, 1976). Previously studies by D'Andrea et al., (1989) had reported that the platelet content of glutamate and aspartate was increased in patients subject to migraine with aura, during headache-free periods, when compared with normal controls and migraine patients without aura. The glutamate level rose further during headache. Ferrari et al., (1990) measured these amino acids in plasma and found the level to be elevated in migraine patients between attacks, more so in those patients whose migraine was accompanied by an aura, and to increase further during headache. In contrast, Alam et al., (1998) showed that the neuroexcitatory, glutamic acid, was elevated in both migraine with and without aura. Martinez et al (1993) found that the levels of aspartate and glutamate in plasma and cerebrospinal fluid (CSF) were higher in migraine patients as opposed to controls. It has therefore been suggested that migraine patients may be predisposed to central neuronal hyperexcitability leading to labile responses (Martinez et al.,

1993). If a similar elevation of excitatory amino acid was shown to exist in the cortex, it would also increase its excitability.

8. Other Hypotheses

Many theories have been proposed to explain migraine pathogenesis. Alterations of neurotransmitter systems (e.g. glutamate, opioids) or anatomical structures (e.g. the raphe system, vasculature) or the autonomic nervous system may be either primary or secondary factors in the evolution of a migraine attack.

ANATOMY OF CENTRAL NERVOUS SYSTEM PATHWAY RELATED TO HEAD PAIN

The fifth (trigeminal) cranial nerve is responsible for conveying sensory information from most of the face and head to the central nervous system. Trigeminal afferents terminate in the brainstem structure known as the trigeminal brainstem nuclear complex (TBNC). The TBNC is composed of the principal (or main) sensory nuclear and spinal trigeminal nucleus. The spinal trigeminal nucleus is further subdivided into a rostral subnucleus oralis, a middle subnucleus interpolaris, and a caudal subnucleus caudalis. Because of its anatomical and physiological similarities to the spinal dorsal horn, subnucleus caudalis is often identified as the medullary dorsal horn (MDH). Earlier in this century, accumulating clinical evidences led a number of investigators to conclude that subnucleus caudalis was responsible for processing pain and temperature information from the face and head. As a result of these observations, the Swedish neurosurgeon, Olof Sjoqvist (1938), developed a trigeminal tractotomy operation for the relief of facial pain. He transected the trigeminal spinal tract at the level of the obex in patients suffering from major trigeminal neuralgia. Following the operation, the patients were usually relieved of the neuralgia (at least temporarily) and reported an almost complete loss of pain and temperature sensibility in the areas supplied by the trigeminal nerve. These clinical data have subsequently been supplemented and extended with a large body of neurophysiological evidence that leaves little doubt regarding the importance of the MDH in the perception of pain. Recent research indicates that trigeminal necleus caudalis (TNC) is not simply a relay station between the face and the thalamus. It has been suggetsed, for

example, that the MDH subserves facial and cranial nociception by a specific modulation of neuronal firing in trigeminal nucleus principalis and oralis (Kruger and Young, 1981). The perception of headache, therefore, may depend more on the projections to TBNC subnuclei other than those on the projections to the thalamus.

Brainstem Terminations of Trigeminal Afferents

The central processes of trigeminal primary afferents enter the brainstem in the sensory root of the trigeminal nerve. Some axons in the sensory root dichotomize into ascending and descending branches. The ascending fibers terminate in the rostral portion of the principalis nucleus, while the descending fibers make up the trigeminal spinal tract. As they descend, axon in the trigeminal spinal tract send collaterals and terminal fibers into the trigeminal spinal nuclei. Numerous anatomical and electrophysiological studies have convincingly demonstrated that the principalis nucleus, the spinal trigeminal tract, and the spinal trigeminal nuclei are topographically organized (Shiginaga et al., 1986). It is clear that mandibular afferents terminate in the dorsal aspect of each trigeminal subnucleus (dorsomedial in the MDH), that ophthalmic afferent terminals are ventral (ventrolateral in MDH), and that maxillary terminals are interposed (Arvidsson, 1982). The results of early anatomical (Kerr, 1963) and neurophysiological (Kruger et al., 1961) studies suggested that each TBNC subnucleus received information from all portions of the face and head. Careful examination of the sequelae of medullary tractotomy procedures, however, later indicated that caudal tractotomies tended to spare sensation in the central portion of the face near the midline (Kunc, 1970). Hayashi (1985), injected HRP into functionally identified primary afferent fibers in the cat's spinal trigeminal nucleus to examine

the central terminations of these labeled axons. He concluded that the collaterals of high-threshold mechanoreceptive afferents formed extensive terminal arbors in the superficial aspect of subnucleus interpolaris and in laminae I and II of caudalis. Renehan (1993) have extended these observations in two intracellular labeling studies in the rat trigeminal system. They found that nociceptive fiber terminations in subnucleus caudalis were focused in two locations, laminae I and II and laminae V. These were resembled the spinal nociceptive fiber terminations described by Light and Perl in 1979. In interpolaris, nociceptive axon arbors were expansive but without a clear terminal focus, except that they often terminated in the medially displaced substantia gelatinosa at the caudal aspect of the nucleus.

Although there is clear evidence that nociceptive primary afferents terminate in interpolaris and caudalis, it is still uncertain whether the rostral TBNC receives direct nociceptive afferent input. No laboratory has conclusively shown that nociceptive primary afferents terminate in principalis or oralis. However, nociceptive higher order neurons have been identified in the rostral subnuclei (Dallel, 1990). Behavioral studies have shown that the rostral subnucleus are important in processing noxious information (Young and Perryman, 1984), and nociceptive projections from the rostral subnuclei to the thalamus have been identified (Raboisson, et al., 1989). Moreover recent studies have shown that many neurons in the rostral as well as caudal trigeminal subnuclei respond to stimuli applied to the cerebral vasculature (Davis and Dostrovsky, 1988; and Dostrovsky et al., 1991). However, many investigators have suggested that nociceptors innervating the cranial blood vessels play a major role in mediating cluster and migraine headache (Dostrovsky et al., 1991).

Projections from the TBNC

Intracellular and extracellular tracing studies have identified a large number of projections from the TBNC (Figure 2.2) It is now clear that the ventrobasal complex of the mammalian thalamus receives direct input from each of the TBNC subnuclei (Huang, 1989; Kemplay and Webster, 1989). There are important differences in the nature and magnitude of the projections. Between 60% and 80% of the TBNC neurons projecting to the contralateral thalamus are in principalis (Kemplay and Webster, 1989). Most of the remaining trigeminothalamic neurons are in subnucleus interpolaris, with smaller contributions from oralis and caudalis. The ipsilateral and contralateral trigeminothalamic projections from principalis terminate in the medial subnucleus of the thalamic ventroposterior complex (VPM) (Kaas, 1990). Recent work by Rhoades et al., 1987 indicates that the receptive fields of VPM neurons are dependent primarily on the input from principalis.

Interestingly, Yokota and Matsumoto (1983), have reported that the responses of these nociceptive VPM neurons can be inhibited by reversibly cooling the caudal TBNC or transecting the spinal trigeminal tract at the level of the obex. This would suggest that the nociceptive projection to the VPM, whatever its importance in actual pain perception, was derived from the caudal TBNC.

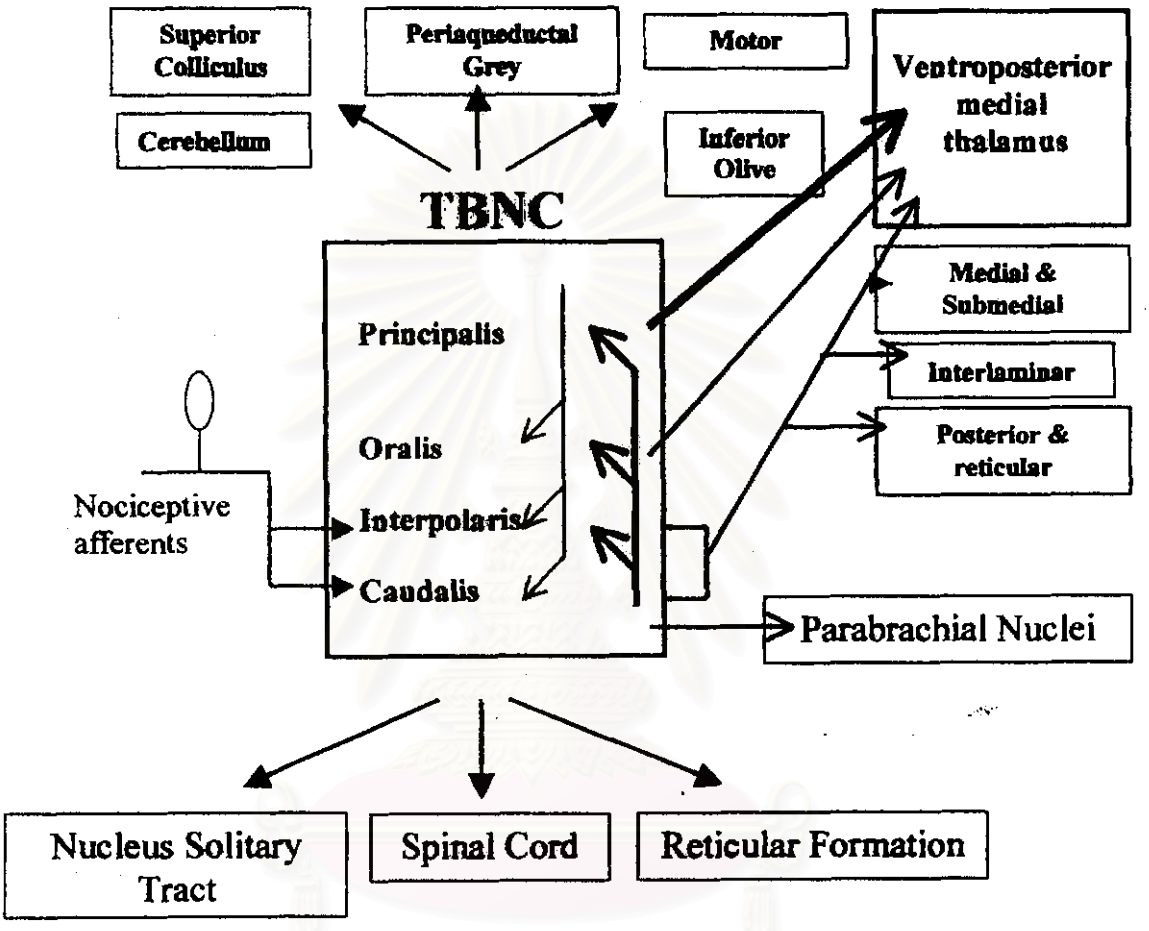

Figure 2.2. Diagram illustrating the principal connections of the trigeminal brainstem nuclear complex (TBNC) thought to be important in nociception. (From Renehan et al., 1986)

In addition to the projection to VPM, the caudal TBNC projects to a number of additional thalamic nuclei. It is these projections that may be most important in the processing of cranial and facial pain. Peschanski (1984); and Shigenaga et al., (1983) have shown that the caudal TBNC has a prominent projection to the posterior thalamus, a region long considered to be important in nociception. Ganchrow (1978) has demonstrated a projection from the caudal TBNC to another thalamic region associated with pain, the internal medullary lamina. Craig and Burton (1981) were the first to demonstrate convincingly a substantial bilateral projection from superficial laminae of the spinal and medullary dorsal horns to the medial thalamus. Using anterograde HRP and autoradiographic techniques, they were able to show that the lamina I cells of the MDH terminate in the caudal aspect of the nucleus submedius in the cat. Dado and Giesler (1990) and Yoshida et al., (1991) have recently demonstrated a similar projection from caudal interpolaris and rostral caudalis in the rat. The dense projection from lamina I to nucleus submedius suggests that the trigeminosubmedial pathway may be important in nociception.

Tract tracing studies have demonstrated TBNC projections to a number of subcortical nuclei in apart from thalamus. The superior colliculus, for example, receives projections from the entire TBNC in the cat (McHaffie et al., 1986) and rodent (Bruce et al., 1987). Huerta et al., (1983) and Jacquin et al., (1989) have noted a projection from interpolaris to the contralateral inferior olive. Matsushita et al., (1982) have noted a projections from interpolaris, caudalis and oralis to cat spinal cord but no trigeminospinal projection from principalis. The TBNC has also been shown to project to the nucleus of the solitary tract (Menetray and Basbaum, 1987). This pathway may be important in the coordination of

somatic and visceral reflexes. Numerous investigators have demonstrated that the neurons in the TBNC project to the adjacent reticular formation (e.g. Renehan et al., 1986) and this connection provides yet another possible substrate for the processing of nociceptive information. In addition to potentially important projections to other brainstem nuclei, that reticular formation has its own projection to the thalamus. Thus neurons that may not have a direct projection to the thalamus may still influence thalamic activity via an "indirect" reticular pathway (Sessle, 1986).

THE TRIGEMINOVASCULAR SYSTEM

Anatomical tracing (Mayberg et al., 1981) and immunohistochemistry studies (Liu-Chen et al., 1983) in laboratory animals (including primates and man) provide unequivocal evidence for the existence of sensory axons innervating cerebral blood vessels. Neuropeptides such as SP (Liu-Chen et al., 1983; Mayberg et al., 1984), neurokinin A (Saito et al., 1987), CGRP (McCulloch et al., 1986) are synthesized by mRNA and ribosomal mechanisms within trigeminal ganglia cells. Neuropeptides are transported from cells within the first division to a plexus within the adventitial layer of cranial vessels. At the electron microscopic level, this plexus consists of small diameter unmyelinated axons characteristic of C fibers (Liu-Chen et al., 1986 and Matsuyama et al., 1985). Neuropeptide-containing vesicles have been visualized within these axons. Neuropeptide release occurs predominantly from these vesicles by calcium-dependent mechanisms (Moskowitz et al., 1983).

In all likelihood, trigeminovascular fibers reach the internal carotid artery at the pericarotid plexus within the cavernous sinus and pierce the dura mater to enter the middle cranial fossa along with the carotid artery. Within the cranium, trigeminal axons distribute predominantly to the ipsilateral anterior, middle, and posterior cerebral arteries. A few fibers cross to innervate the contralateral anterior cerebral artery (Moskowitz et al., 1993a and O'Connor et al., 1986). Recent studies indicate that trigeminovascular fibers project widely within the cranium, so that, for example, the same trigemimal ganglia cell that projects to the middle cerebral artery also sends an axon to innervate the middle meningeal artery (O'Connor et al., 1986). The density of sensory axons is greatest along the proximal arteries of the circle of Willis and diminishes

considerably over the convexity. Recently, SP and CGRP-containing neurons were found in a satellite miniganglions located on the carotid artery within the carotid canal. Neurons within this ganglion send axonal projections to innervate large pial arteries (Suzuki et al., 1988). This finding explains why total removal of the trigeminal ganglia in experimental animals does not completely deplete the pial vessels of their sensory neuropeptides. The relationship of this miniganglion to vascular headaches remains to be explored.

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.

Neocortex as a Potential Migraine Trigger

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

(Moskowitz et al., 1993b) (Figure 2.3). In this study, the effects of neocortical spreading depression were examined on the expression of immunoreactive Fos protein within the superficial laminae of TNC. To induce recurrent CSD, KCl was microinjected repeatedly into the left parietal cortex; CSD was detected by electrophysiological methods within adjacent frontal cortex. In response, Fos-like protein was visualized in the ventrolateral TNC (corresponding to the ophthalmic division), chiefly in lamina I, II_0 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).

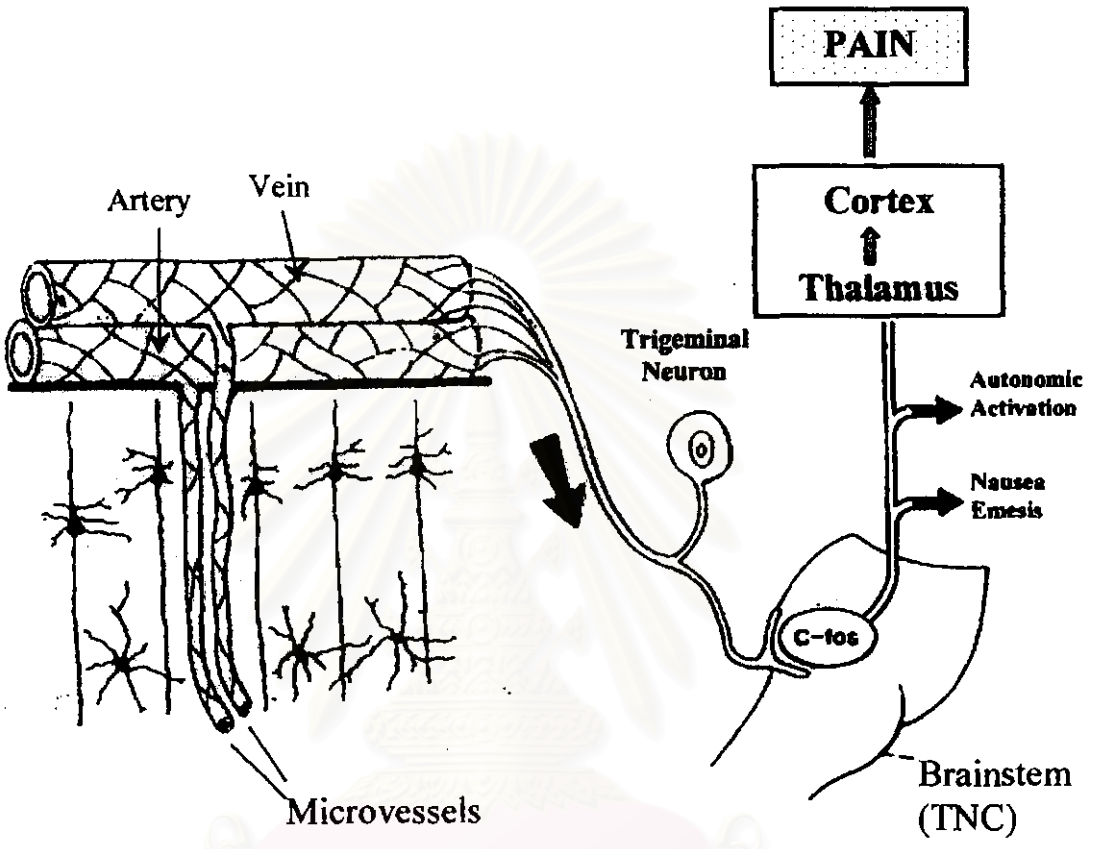

Figure 2.3. Diagram depicting the relationship between pial vessels, the trigeminovascular system, and neocortex. Recent data indicate that neurophysiological activity within neocortex (i.e. recurrent spreading depression) can activate the ipsilateral trigeminovascular system, as evidenced by induction of the c-fos antigen within lamina I, II_O 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 , 1993c)

The findings are consistent with the following formulation: neurophysiologically driven ionic and metabolic mechanism (e.g. CSD) promote the release of nociceptive substances from neocortex into the interstitial space. Within the perivascular space, released substances activate and sensitize trigeminovascular fibers surrounding pial vessels. As a result, impulses are conveyed to TNC and other higher center, resulting in pain sensation. Pain may ensue. One speculate that these findings may be relevant to headache pathophysiology and to the pathogenesis of photophobia and phonophobia accompanying stimulation of meningeal afferents.

THE SOURCE OF PAIN IN MIGRAINE HEADACHE

Migraine headache often starts as a dull pain in the frontotemporal region, upper neck or occipital area and becomes pulsatile in character only as the severity of the attack increases. The development of pain in migraine headache may be attributed to both vascular and neuronal mechanisms.

VASCULAR MECHANISMS

Because of the throbbing quality of the pain, the conspicuous dilatation of cranial arteries has long been considered.

The Extracranial Circulation

Graham and Wolff (1938) recorded the pulsation of branches of the superficial temporal artery during migraine headache and observed that the amplitude of the pulse wave declined as the intensity of headache diminished after the injection of ergotamine tartrate. The concept of

migraine being an "*extracranial vascular headache*" appeared to be strengthened by the studies of Tunis and Wolff (1953) which showed that the mean amplitude of temporal artery pulsations was greater during headache than in periods of freedom.

Sakai and Meyer (1978) found that extracranial blood flow increased by about 20 per cent on the side affected by headache. Jensen and Olesen (1985) reported that temporal muscle blood flow was increased by about one-third during headache, however, this change did not reach statistical significance and there was no difference between headache and non-headache sides. Nevertheless, arteries and veins do become prominent in the temple during migraine, and pressure over them eases the pain.

Blau and Dexter (1981) assessed the contribution of extracranial arteries to migraine headache by inflating a sphygmomanometer cuff around the patient's head. Of 47 patients, only 21 experienced relief from headache after inflation of the pericranial cuff. Drummond and Lance (1983) compared the pulse amplitude of the superficial temporal artery and its main frontotemporal branch with the intensity of pain felt in the temple while the ipsilateral common carotid and temporal arteries were compressed alternately. Of 62 patients, selected only by the presence of a unilateral migrainous headache, the pain appeared to be of extracranial vascular origin in about one-third, was of mainly intracranial vascular origin in one-third, and had no detectable vascular component in the remaining one-third. In the subgroup with increased arterial pulsation in the frontotemporal region, thermography demonstrated increased heat loss from this area, and temporal artery compression eased the headache briefly. Although the frontal branches of the temporal artery were found to dilate in one-third of patients, Drummond and Lance (1983) could not

detect any change in the pulsation of the superficial temporal artery itself. Iversen et al., 1990 demonstrated by Doppler studies that the lumen of the temporal artery is increased during ipsilateral migraine headache relative to that of the opposite side and the lumen of peripheral arteries.

The Intracranial Circulation

Ample evidence indicate that migrainous headache does not depend solely on cranail vasodilatation. Migraine without aura is not usually associated with alteration of regional cerebral blood flow (Olesen et al., 1981b) and the headache of migraine with aura usually starts while blood flow is still reduced (Olesen et al., 1990). Kobari et al., (1989) found that cerebral blood flow was increased during migraine headache but this increase was not related to the side on which headache was experienced. Geraud et al., (1989) described areas of hypoperfusion (clinically silent oligaemic areas) as well as regions of hyperperfusion co-existing in patients with common migraine without aura.

Transcranial doppler sonography has recently been used to assess the velocity of flow in proximal branches of the internal carotid artery. Friberg et al., (1991) found that flow in the middle cerebral artery was reduced on the side affected by headache. Since regional cerebral blood flow in the territory supplied by that artery was unaltered, they suggested that the lower velocity was caused by dilatation of the middle cerebral artery. Following the intravenous infusion of 2 mg of sumatriptan, the headache relieved and the velocity of flow in the middle cerebral artery returned to normal within 30 minutes. These observations are reminiscent of those made by Graham and Wolff (1938) concerning the effect of ergotamine on extracranial arteries. They indicated that vascular

dilatation accompanies some migraine attacks but it does not follow necessarily that the distended vessels are entirely responsible for headache. Diener et al., 1991 could not demonstrate any change of blood flow velocity in the extracranial portions of the internal or external carotid arteries, or in the middle cerebral or basilar arteries, after the injection of sumatriptan 4 mg subcutaneously.

Moskowitz (1992) has pointed out that sumatriptan and the ergot alkaloids attenuate the release of neuropeptides from trigeminovascular fibers and may thus play a wider role than vasoconstriction by reducing the "*sterile inflammatory response*" of migraine headache. The level of vasodilator substance, CGRP in the jugular venous blood, which is elevated during migraine headache, return to baseline after the headache is relieved by sumatriptan (Goadsby and Edvisson, 1992).

Muscle Contraction

Pain from muscle contraction may add a non-vascular component to migraine headache. Excessive contraction of the temporal, masseter, and neck muscles is common in migraine patients, more than in patients with "*tension headache*" (Lous and Olesen, 1982). Becomes evident just before the headache reaches its maximum (Bakke et al., 1982). Tfelt-Hansen, Lous and Olesen (1982) found that infiltration of tender muscle areas with local anaesthetic or normal saline 70 minutes relieved migraine headaches in 28 of 48 patients. 5-HT and bradykinin potentiated the pain-producing effects of one another when injected into the temporal muscle of normal volunteers but did not evoke headache (Jensen et al., 1985).

THE CENTRAL NERVOUS SYSTEM

Migraine may be regarded as a hereditary sensitivity of neurovascular reactions to certain stimuli in the central nervous system. Raskin and Knittle (1976) found that cold drinks or ice cream evoked headache in 93 per cent of migraine patients compared with only 31 per cent of control subjects. One-third of patients with ice-cream headache state that this pain involves precisely the same part of the head as their habitual migraine headaches (Drummond and Lance, 1984). Moreover, 42 per cent of migraine patients are prone to sudden jab of pain in the head (ice-pick pain), compared with 3 per cent of non-headache controls (Raskin and Schwartz, 1980). Drummond and Lance (1984) found that ice-pick pain coincided with the site of the customary headache in 40 per cent of patients. The trigeminal pathways may thus become activated spontaneously in paroxysms lasting a fraction of a second (ice-pick pain) or may be activated reflexly for seconds or minutes by sudden cooling of the pharynx (ice-cream headache). This indicates a persisting disinhibition of segment of the trigeminal pathways in migraine patients, suggesting that the trigeminal system could also discharge excessively for hours or days to provide a neural origin for migraine headache.

Based on the above, it seems intuitively correct that, despite the lack of exacting information regarding headache pathogenesis, headache pain should ultimately be resulted from the activation of the trigeminovascular system. Accordingly, the perivascular primary sensory neuron may be viewed as a final common pathway subject to activation or modulation by local factors within the vessel lumen and vessel wall. Elucidating these factors will provide both excitement and challenge for future investigators in this field and possibly therapeutic benefits for migraineurs.

REVIEW OF THE ROLE OF NITRIC OXIDE IN MIGRAINE HEADACHE

In 1980 Furchgott and Zawadzki reported that vasodilatation induced by acetylcholine depends on the presence of intact endothelium. 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.

BIOLOGY OF NO

The highly reactive free radical NO is a lipophilic gas of formula N=O. It is a unique molecule as far as endogenously formed regulatory substances are concerned. Since NO is a very small lipophilic molecule, it can rapidly diffuse through biological membrane barriers and thereby reach the intracellular compartments of nearby cells of diverse function. Interestingly, much like oxygen, NO is actually a gas that is sparingly soluble in aqueous medium and functions biologically as a molecule in solution. The ultrashort half-life of NO (probably in the range of 5-30 second in biological tissues) limits the availability of newly synthesized of NO to adjacent or nearby cells. Under physiological conditions of neutral pH, NO is not very reactive, but its paramagnetic property is responsible for its remarkably high binding affinity for heme iron. Thus, NO reacts with numerous hemoproteins (reduced Fe^{2+} state) to generate their nitrosylheme adducts, and these are relatively more stable than free NO.

The most important hemoprotein interaction involving NO is that of the reaction between soluble guanylate cyclase (sGC) and NO to yield the nitrosyl-heme-enzyme tertiary complex, which represents the activated state of guanylate cyclase. The binding of NO to the heme group of guanylate cyclase causes an immediate and profound increase in catalytic activity, resulting in a 50-fold to 200-fold increase in the velocity of conversion of magnesium guanosine 5'- triphosphate (MgGTP) substrate to cyclic guanosine 3',5'- monophosphate (cGMP). This interaction between NO and the heme group of guanylate cyclase represents a novel and widespread signal transduction mechanism that links extracellular stimuli to the boisynthesis of the second messenger cGMP in adjacent cells.

CHEMISTRY

The biochemistry of nitrogen monoxide involves an array of interrelated redox forms. Under physiological conditions there is interconversion among the different redox forms (Stamler et al., 1992). The redox form normally referred as NO, or simple NO, is the highly reactive free radical $NO^{\cdot}$ (NO dot), an inorganic gas of formula $^{\cdot}N{=}O$ (Kiechle and Malinski, 1993). In this neutral redox form, NO is poorly soluble in water and easily penetrates biological membranes (Kiechle and Malinski, 1993). Under physiological conditions (i.e. in an oxygenated and heated aqueous solution with pH around 7.4, 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). In whole blood, NO is rapidly converted to nitrate by its reaction with oxyhemoglobin (Kelm et al., 1992). NO reacts with intracellular iron pools and thus binds to the heme moiety of different heme-

containing proteins (e.g. hemoglobin, myoglobin, cytochrome C and sGC) (Reif and Simmons, 1990). Furthermore, NO binds to sulfhydryl groups (SH groups) and yields thionitrites or S-nitrosothiols (Stamler et al., 1992). Thiols (R-SH) are present in a variety of protein and amino acid compounds, and it has been suggested that thiol groups function as intracellular storage and/or transport molecules for NO (Venturini et al., 1993). NO also readily reacts with superoxide to form the unstable and toxic metabolite peroxynitrite. The effects of NO are, therefore, prolonged when O_2^- is degraded by superoxide dismutase (Gryglewski et al., 1986).

SYNTHESIS

In biological systems, NO is generated from the terminal guanidino nitrogen of L-arginine. This oxidative reaction requires NADPH, O_2, flavins (FMN and FAD) and tetrahydrobiopterin (BH_4) and yields citrulline as a co-product (Mayer, 1994)(Figure 2.4). The transport of L-arginine within cells involves cationic amino acid transport systems (Greene et al., 1993). Under normal physiological conditions the supply of L-arginine seems not to be the rate-limiting step (Mayer, 1994). The enzymes responsible for NO synthesis are known as NOS. NOS activity has been reported in many tissues, including endothelium, brain, peripheral nerves, vascular smooth muscle, myocardium, macrophages, neutrophils and microglia of several species (Knowles and Moncada, 1994; Forstermann et al., 1994). Purification and cloning of NOS genes has revealed the existence of at least three isoforms of NOS (Knowles and Moncada, 1994)(Table 2.2). Two of these are constitutive, Ca^{2+}/calmodulin-dependent and release NO from, for example, endothelium (eNOS) and neurons (nNOS). Another NOS is inducible

NOS (iNOS) and generally Ca^{2+} independent. After induction of iNOS, NO is released from macrophages, astrocytes, microglia and vascular smooth muscle cells for long periods and in large amounts in response to, for example, endotoxins and cytokines (Busse and Mulsch, 1990; Wallace and Bisland, 1994) (Figure 2.5). The chromosomal location of nNOS, eNOS and iNOS genes has been described in human-rodent hybrid cell lines. The nNOS gene maps to human chromosome 12 (Xu et al., 1993), the eNOS gene map to chromosome 7 and iNOS gene map to chromosome 17 (Xu et al., 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.

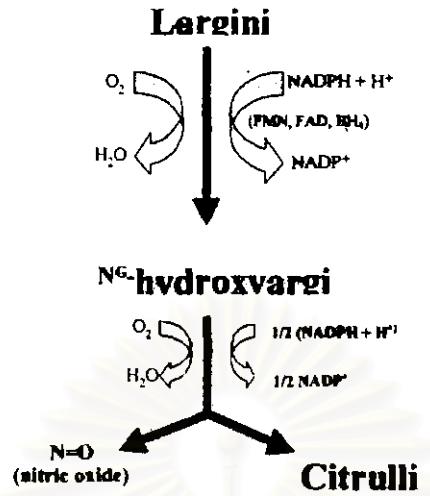

Figure 2.4. Synthesis of NO from L-arginine. This two-step oxidation requires NADPH, O_2 flavin (FMN and FAD) and tetrahydrobiopterin (BH_4) as co-factors and yields citrulline as a co-product. (From Mayer, 1994)

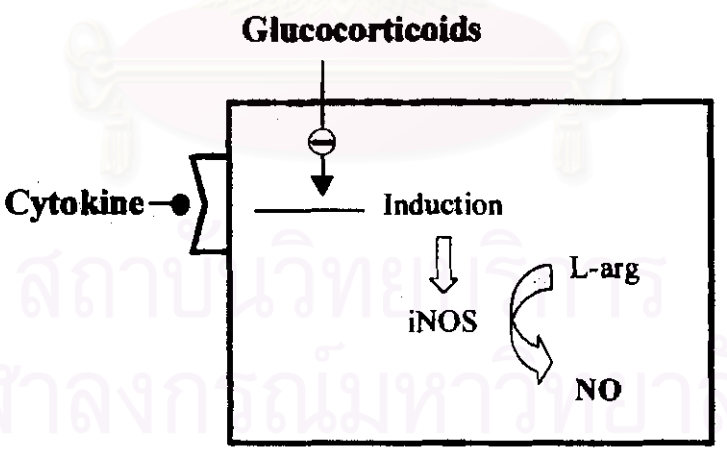

Figure 2.5. Induction of inducible NO synthase (iNOS) by for example cytokines may be inhibited by glucocorticoids and by a variety of L-arginine analogue. The later also inhibit eNOS and nNOS. (From Wallace and Bisland, 1994)

Stimulation and Inhibition of NOS

The regulation of the NOS is not fully understood. It is clear that iNOS can be induced by stimulation with cytokines or endotoxins and that eNOS and nNOS are stimulated by an increase in intracellular Ca^{2+} (Knowles and Moncada, 1994). Stimulation of several specific membrane-bound receptors by, for example, glutamate (NMDA receptor), bradykinin (B_2-kinin receptor), 5-HT (5-HT_{2C} receptor), acetylcholine (muscarinic M_1 or M_3 receptor), histamine (histamine H_1 receptor), endothelin-1 (ET_{1B} receptor) and SP increases eNOS and nNOS activity (Garthwaite, 1991; Namaki et al., 1992; Murphy et al., 1993; Glusa and Richter, 1993; Carmignani et al., 1997) (Figure 2.6). Increase blood flow velocity and the subsequent increase of shear stress in endothelial cells may also stimulate eNOS (Buga et al., 1991; Fostermann et al., 1994). Estrogen increases both eNOS and nNOS activity probably via stimulation of transcription of the genes (Knowles and Moncada, 1994). In the vascular smooth muscle cells there may be a positive feedback mechanism, since cGMP, which is increased by NO, upregulates iNOS expression (Inove et al., 1995). However, NO may also be inhibited by NO itself as a result of feed back mechanisms (Rogers and Ignarro, 1992; Assreuy et al., 1993; Yeh et al., 1996)

BIOLOGICAL EFFECT OF NO

Most biological actions of NO are mediated via binding to the heme moiety of sGC, hence causing a consequent increase in cGMP and eventually leading to a decrease in intracellular Ca^{2+} in target cells. (Moncada et al., 1991; Garthwait et al., 1991) (Figure 2.6)

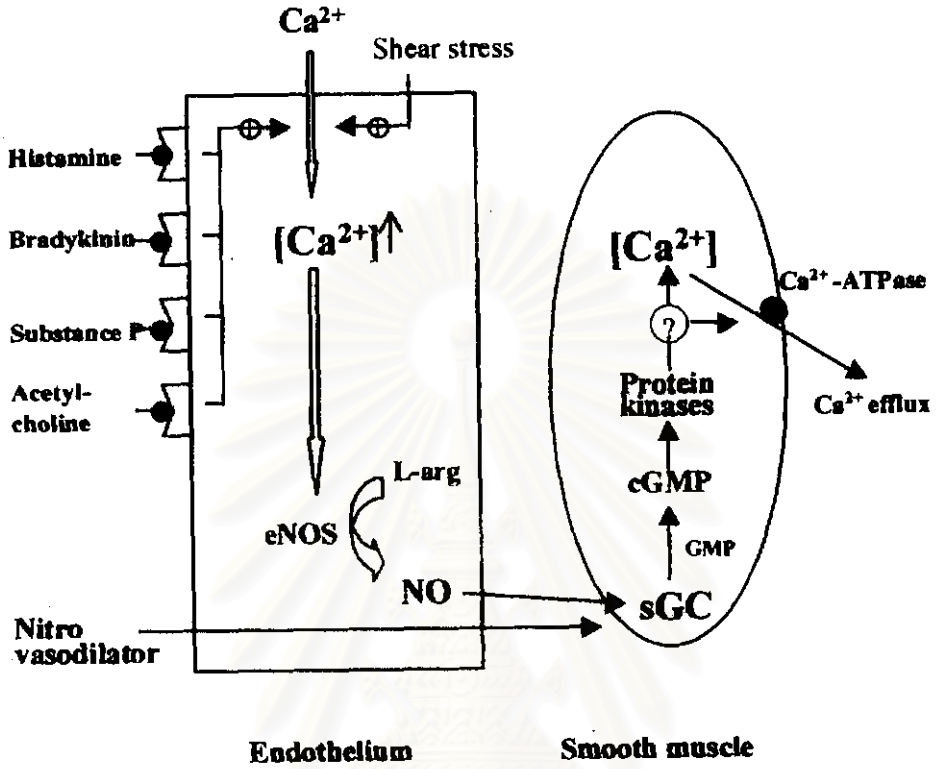

Figure 2.6. Modular events in the NO pathway illustrated by endothelium-derived synthesis of NO. Endothelium nitric oxide synthase (eNOS) is stimulated by an increase in intracellular calcium. This increase may be caused by shear stress and receptor stimulation (e.g. histamine, bradykinin, substance P and acetylcholine). NO diffuses from endothelial cells to smooth muscle cells and activates the soluble guanylate cyclase (sGC). This in turn leads to an increase in cyclic guanosine monophosphate (cGMP) and via activation of protein kinases and subsequent poorly understood intermediately processes stimulates the membrane bound Ca^{2+} ATPase. Ca^{2+} then diffuses out of the cell, eventually leading to smooth muscle relaxation and vasodilatation. Nitrovasodilators act as NO donors and activate the same pathway. (From Garthwaite, 1991)

sGC is also activated by arachidonic acid, polyunsaturated fatty acid and/or their hydroxy peroxy derivatives, superoxide, hydroxyl radicals, thiols and carbon monoxide (CO)(Zhuo et al., 1994). Methylene blue has been used as a specific inhibitor of sGC in several experimental situations; however, this is controversial since it is likely that this compound inhibits the NO-cGMP pathway by producing superoxide (which inactivates NO) rather than by inhibition of guanylate cyclase (Marczin et al., 1992). The various molecular targets of cGMP include (i) activation of specific protein kinases, which in turn catalyze phosphorylation of different enzymes and other proteins, (ii) inhibition of the generation of inositol triphosphate (IP_3) (iii) increased sequestration of cytosolic Ca^{2+} , (iv) inhibition of Ca^{2+} influx, (v) stimulation of membrane Ca^{2+}-ATPase, (vi) opening of K^+ channels, (vii) dephosphorylation of the light chain of myosin, and (viii) inhibition of a cGMP regulated cAMP phosphodiesterase (Figure 2.7) (Goy, 1991; Schmidt et al., 1993; Faraci et al., 1998). Of these, cGMP stimulated protein kinases appear to be the predominant mediators for the cardiovascular and neuronal effects of NO (Zhuo et al., 1994; Walter, 1989) (Figure 2.7). Numerous proteins are in turn likely to be phosphorylated by cGMP-stimulated protein kinases. The most important final effector that mediates the cGMP-stimulated reduction in intracellular Ca^{2+} (at least in smooth muscle cells) seems to be the plasmalemmal Ca^{2+} ATPase. The breakdown of cGMP is a tightly controlled process regulated by a large number of different phosphodiesterases (PDEs), which again are tightly regulated. PDEs have been classified within seven families, PDE I-PDE VII, with different affinities for cGMP and cAMP (Eckly and Lugnier, 1994). The extent of cGMP hydrolysis is dependent upon the pattern of PDE isoforms expressed in different tissues. It is of interest to note that not only is cGMP hydrolyzed by specific PDEs, it also contributes to the regulation

(stimulation and inhibition) of the activities of other PDEs preferentially catalyzing the hydrolysis of cAMP. Thus, the PDEs may be involved in a complex cGMP-cAMP relationship (Eckly and Lugnier, 1994). To this end, and increase in endothelial cAMP as a result of stimulation with beta-2 agonists and CGRP facilitated NO production in endothelial cells in isolated rat aorta segments (Gray, 1992a and 1992b). Thus, at least in certain situations, cAMP may act synergistically to cGMP in the relaxation of vascular smooth muscle.

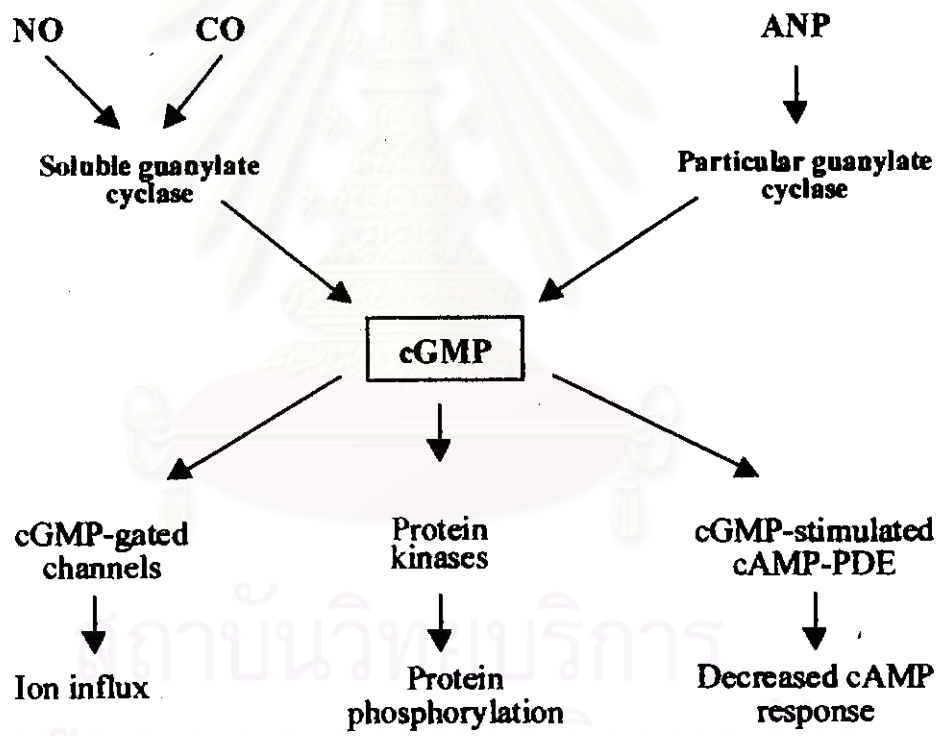

Figure 2.7. Synthesis of cGMP is catalyzed by guanylate cyclase. A particulate and a soluble form exists. The particulate form is stimulated by for example, atrial natriuretic peptide (ANP). The soluble form is stimuated by for example NO and carbon monoxide (CO). cGMP in turn activates intracellular processes, e.g. ion influx, protein phosphorylation and/or phosphodiesterases (PDE). (From Schmidt et al., 1993)

Targets of NO Toxicity

The toxic actions of NO are thought to be mediated by NO reacting with iron containing enzymes in target cells, eventually leading to inhibition of mitochondrial respiration and DNA synthesis (Stamler et al., 1992). NO also contributes to deamination-related genetic mutations (Dawson et al., 1992). Alternatively, NO can react with the superoxide anion to form peroxynitrite, which is an extremely reactive molecule with strong oxidant properties (Stewart et al., 1994). Furthermore, once peroxynitrite is protonated it decomposes to the hydroxyl-free radical, which is also a highly reactive and potentially damaging molecule, and to nitrogen dioxide, which is a potent activator of lipid peroxidation (Dawson et al., 1992) (Figure 2.8)

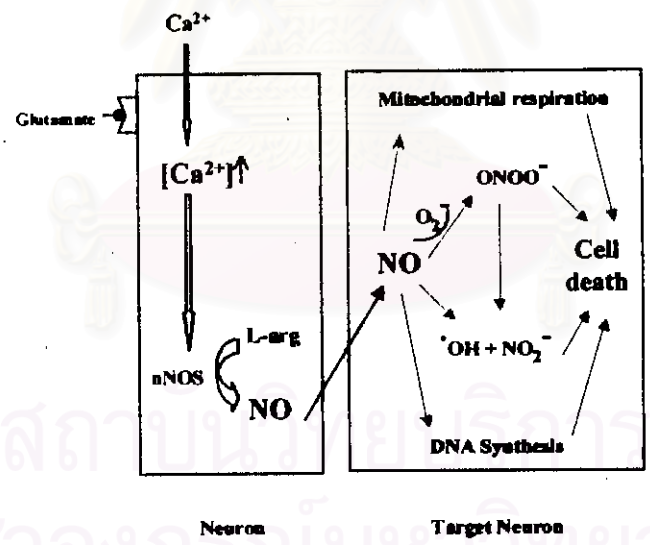

Figure 2.8. Neurotoxic effects of NO. Excessive NO is formed during sustained glutamate stimulation of NMDA receptors. NO diffuse to adjacent neurons (named "target neurons") where it produce peroxynitrite ($ONOO^-$), inhibits mitochondrial respiration and interferes with DNA synthesis eventually leading to cell death. (From Dawson et al., 1992)

Evidence Supporting a Role of NO in Migraine Pain

One way of testing whether a given molecule is involved in a suspected process is by means of provocation with the substance. The short half-life of NO, and the potential toxicity when produced or given in excess, makes it difficult to administer NO directly in clinical studies. Instead, administration of a NO donor may provide some answers. The nitrovasodilators are a diverse group of pharmacological agents which produce vascular relaxation by releasing NO and, therefore, activating sGC (Axelsson et al., 1979; Feelisch and Noack, 1987) (Figure 2.6). The mechanisms by which these compounds release NO vary. Sodium-nitroprusside, nitrosamines and nitrosothiols release NO non-enzymatically, whereas organic nitrates release NO via an enzymatic process which is not fully understood (Ignarro, 1989; Feelisch, 1993; Harrison and Bates, 1993). *In vivo*, organic nitrate esters may interact with free thiols such as cysteine in cells to generate labile intermediate s-nitrosothiols that spontaneously liberate NO (Ignarro, 1989; Harrison and Bates, 1993). This group of compounds includes NTG, which has been used safely in the treatment of angina pectoris for more than a century.

NTG are substances which reliably and dose dependently produce headache in normal volunteers and migraine sufferers (Krabbe and Olesen, 1980; Iversen et al., 1989a; Olesen et al., 1993). NTG itself has no known action in the human body but acts via liberation of NO and is thus generally regarded as a NO donor (Ignarro et al., 1981; Feelisch and Noack, 1987). NTG is the most suitable substance for experimental studies of NO-induced headache since it is well tolerated and diffuses freely across membranes due to its lipid solubility. It may thus deliver NO to several tissues including those protected by the blood brain barrier.

The following observations support that NTG induces headache by liberating NO: (i) NTG induced headache in normal controls is very short lived and is therefore unlikely to be caused by metabolites other than NO, since these have a longer half-life (Iversen et al., 1989a) (ii) the long-acting nitrate 5-isosorbide mononitrate (5-ISMN) induces a dose-dependent headache and arterial dilatation but its metabolites apart from NO are different from those of NTG (Iversen et al., 1992b), (iii) N-acetylcysteine, which augments NTG effects in the heart by increasing the formation of NO or by enhancing the effect of NO itself, also augments the headache response to NTG and prolongs NTG induced arterial dilatation of the superficial temporal artery but not of the radial arteries (Iversen, 1992a). Histamine also seems to induce headache via NO. Thus, in human cerebral blood vessels, histamine stimulates an endothelial H_1 receptor which activates NOS (Toda, 1990; Ottosen et al., 1991). Histamine thus stimulates the endogenous formation of NO whereas NTG delivers NO directly.

NO-induced Headache in Healthy Subjects.

Iversen et al., (1989a) validated the headache-inducing properties of NTG in non-migraineurs and developed a reproducible experimental headache model based on intravenous infusion of NTG and recordings of headache characteristics and intensity. The latter on a 0 to 10 point verbal rating scale. The NTG-induced headache in non-migraineurs was found to be of mild or moderate intensity. Steady state was reached after approximately 10 min of infusion. The headache rapidly disappeared after the NTG infusion was stopped. Headache responses were dose-dependent up to 0.5 μg/kg/min, after which a ceiling effect was observed. The headache in non-migraineurs had some of the features of a migraine

attack (as defined by the diagnostic criteria of the IHS), but differed from migraine by being milder and without nausea, photo- and phonophobia.

NO Hypersensitivity in Migraine

Previous studies have suggested that subjects with a personal history of migraine or a positive family history of migraine more often experience a migraine-like headache in association with NTG administration than do non-migraineurs (Sicuteri et al., 1987). In recently, it has been confirmed in controlled double blind trials that migraineurs with a time delay of several hours (peak intensity 5.5 hours after NTG infusion) actually develop a genuine migraine attack after NTG infusion (Olesen et al., 1993; Thomsen et al., 1994). This migraine headache is preceded by an immediate headache response during the infusion, resembling but not fulfilling diagnostic criteria for migraine without aura (IHS criteria). The immediate headache response, which also is seen in non-migraineurs, is more severe in migraineurs (Olesen et al., 1993). Thus, migraineurs are hypersensitive to NTG induced headache and most likely therefore to NO. An increase headache response could, however, reflect a greater general sensitivity to pain or it could be due to increased physiological sensitivity to nitric oxide. It is well known that NTG dilates the middle cerebral artery via NO without affecting cerebral blood flow (Dahl et al., 1989; Iversen et al., 1989b). Applying the ultrasound technique Transcranial Doppler (Thomsen et al., 1993b), which provides an indirect measure of large intracranial artery diameters in situations of unchanged blood flow, they examined whether the increased sensitivity to the NO donor was reflected not only increased headache in migraineurs but also increased dilatation of the middle cerebral artery. Indeed migraineurs were found to be more sensitive in

this aspect as well. During a 3 hours observation period the time profile of the NTG-induced middle cerebral artery dilatation corresponded with the headache response (Thomsen et al., 1993a and 1994). Thomsen et al., (1993a) compared NTG-induced headache responses in 17 sufferers of migraine without aura, 17 headache-free individuals and 9 patients suffering from episodic tension-type headache. Each person received a staircase intravenous infusion of four increasing doses of NTG and headache responses were followed prospectively until 1 hour after termination of the previous infusion. Migraineurs reported headaches of stronger intensity and with more migraine characteristics than did the controls (Figure 2.9). This difference was most apparent at the higher NTG doses. Retrospectively recorded data from 1 to 24 hours after NTG infusions showed that 11 migraineurs, in addition to the headache experienced in close association with NTG infusions, complained of a "delayed" headache which they labeled as typical migraine (Table 2.3). Some of the controls also experience a delayed headache, but of milder intensity and only in a single case could it be classified as a migraine (Olesen et al., 1993). In a placebo-controlled trial, Thomsen et al., (1994) prospectively recorded information on the delayed headache characteristics and accompanying symptoms in 10 sufferers of migraine without aura until 12 hours after infusion of NTG. These characteristics were compared to the characteristics of spontaneous attacks in the same patients. Eight patients experienced headache during the NTG infusion but only one of these headaches satisfied the migraine criteria. However, eight patients developed a regular migraine attack (fulfilling the IHS diagnostic criteria for migraine without aura) after NTG. Peak migraine headache occurred at a mean of 5.5 hours after the infusion (Figure 2.10). The median peak headache intensity in the eight migraine patients who experienced a post-infusion migraine was 7, ranging from 3 to 10 (0-3

mild, 4-6 moderate, 7-10 severe) and the pain characteristics and accompanying symptoms of the induced migraine attacks were very similar to those reported during spontaneous migraine attacks in the patients (Figure 2.11). Thus, patients suffering from migraine without aura are more sensitive to NTG-induced headache in two ways. First, they experience more severe immediate non-migraine headache than do controls, and, second, most migraineurs, in contrast to controls, eventually experience a delayed migraine. This increased sensitivity to NTG could reflect the increased sensitivity to NO. This view is supported by increased arterial vasodilatation during NTG infusions in migraineurs (Thomsen et al., 1993a). Furthermore, there are the delayed and prolonged cerebral vascular dilatation in migraine patients who develop a delayed headache after NTG infusion compared to controls. Furthermore, the decrease platelet aggregability to collagen in combination with increased platelet arginine levels in migraineurs support that migraineurs are supersensitive to physiological effects of NO (D'Andrea et al, 1994; Gallai et al., 1996). Moreover, migraineurs have been found to be hypersensitivity to histamine regarding headache development in controlled trials (Krabbe and Olesen, 1980; De-Marinis et al., 1990). The headache induced during histamine infusion was almost completely blocked by the histamine H_1-blocker, mepyramine whereas the H_2-blocker, cimetidine only had a small effect (Krabbe and Olesen, 1980). In a recent double blind controlled trial migraineurs were randomized to pretreatment with either mepyramine or placebo before histamine infusion. Half of the placebo-pretreated patients developed, beside the immediate headache during the histamine infusion, a delayed migraine attack fulfilling the IHS criteria for migraine without aura. The peak intensity of this induced migraine attack was reached 5.1 hours after the histamine infusion. The mepyramine pretreated patients only developed a

very mild headache if any (Lassen et al., 1995). Most interesting feature was that the temporal profile of the induced headache was exactly the same as after NTG-infusion. As mentioned above, activation of endothelial H_1-receptors induce the formation of endogenous NO (Toda, 1990). Thus, the increased sensitivity to histamine in migraineurs may also be explained by hypersensitivity to activation of the NO pathway, and activation of this pathway is likely to be a final common pathway for the headache and arterial dilatation induced by both histamine and NTG. Further evidence has recently been supplied by the finding that NTG induced headache could not be prevented by histamine H_1-receptor blocking (Lassen et al., 1996), indicating that the final common mechanism of histamine- and NTG-induced headache is not explained by histamine release after NTG infusion (Iversen and Olesen, 1994). In contrast, NOS-inhibition could be reduced the histamine-induced headache in healthy subjects (Schmetterer et al., 1997) This data supported the concept that the vasodilatation action of histamine depend on NO production.

Table 2.3. Comparison of delayed NTG-induced headache in migraine and control

	Migraine Without aura (n=17)	Tension-type headache (n=9)	Healthy subject (n=17)
No. of subjects with delayed NTG-induced headache	13	7	7
No. fulfilling the IHS criteria for migraine with aura	11	1	0

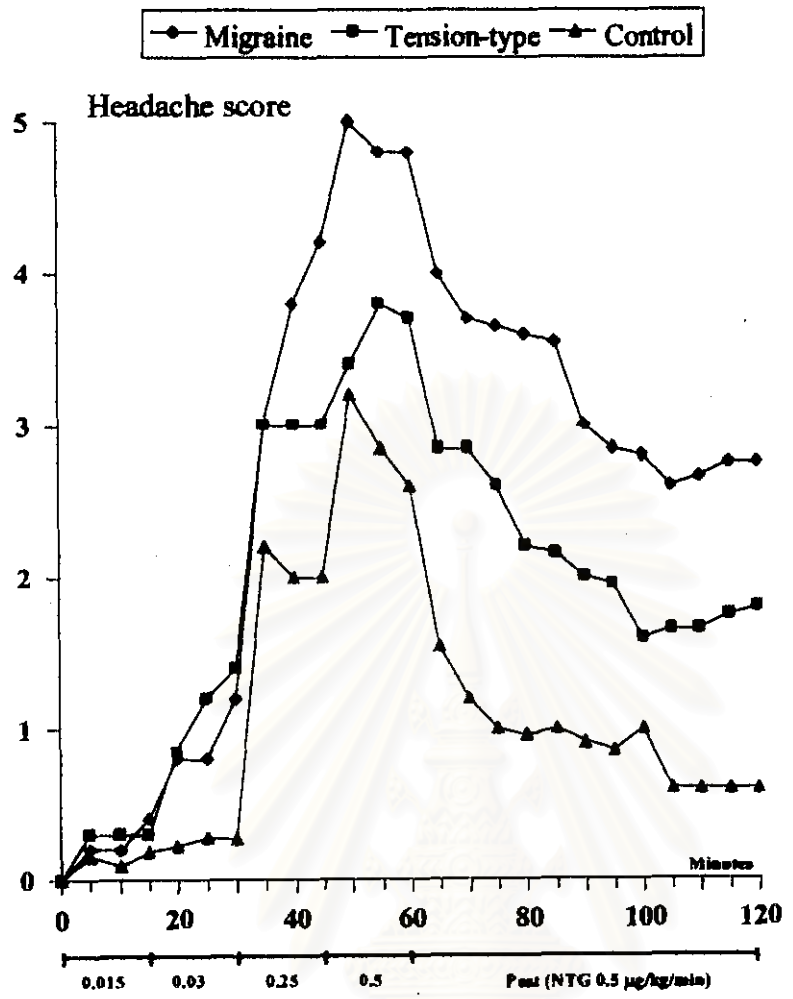

Figure 2.9. Comparison of mean headache intensity (score on a 0-10 point scale) over time during and after four doses of NTG infusion in 17 migraineurs, 9 tension-type sufferers and 17 healthy controls. Headache increase significantly above baseline after 3.5 min in all three groups. During doses above 0.015 µg/kg/min migraine patients experienced significantly more headache than control ($p<0.05$). In migraine patients the headache remained increased during the study period, whereas headache 60 min after and end of the infusion in both controls and tension-type headache patients was no different from baseline ($p<0.001$). (From Olesen J, Iversen HK, Thomsen LL, 1993).

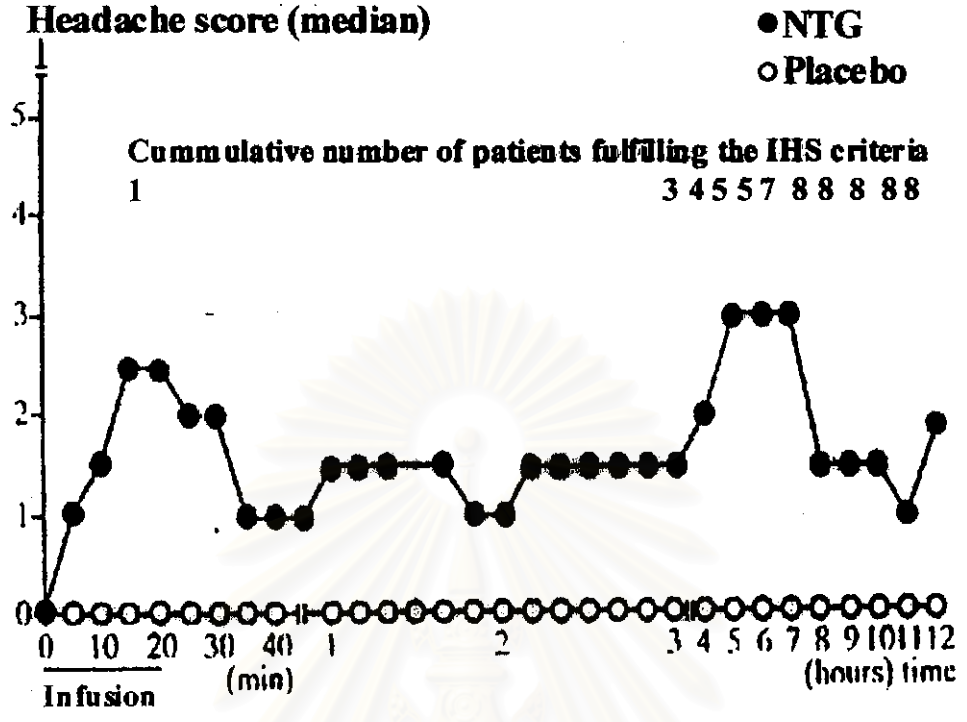

Figure 2.10. Median headache intensity over time during and after NTG and placebo infusion in 10 sufferers of migraine without aura. The headache reached it peak value at a mean of 5.5 h [median peak intensity 7 (range 3-10) in eight patients who experienced a delayed migraine] after termination of NTG infusion. The accumulative number of patients fulfilling the IHS criteria for migraine without aura are shown at the top of the figure. (From Thomsen et al., 1994).

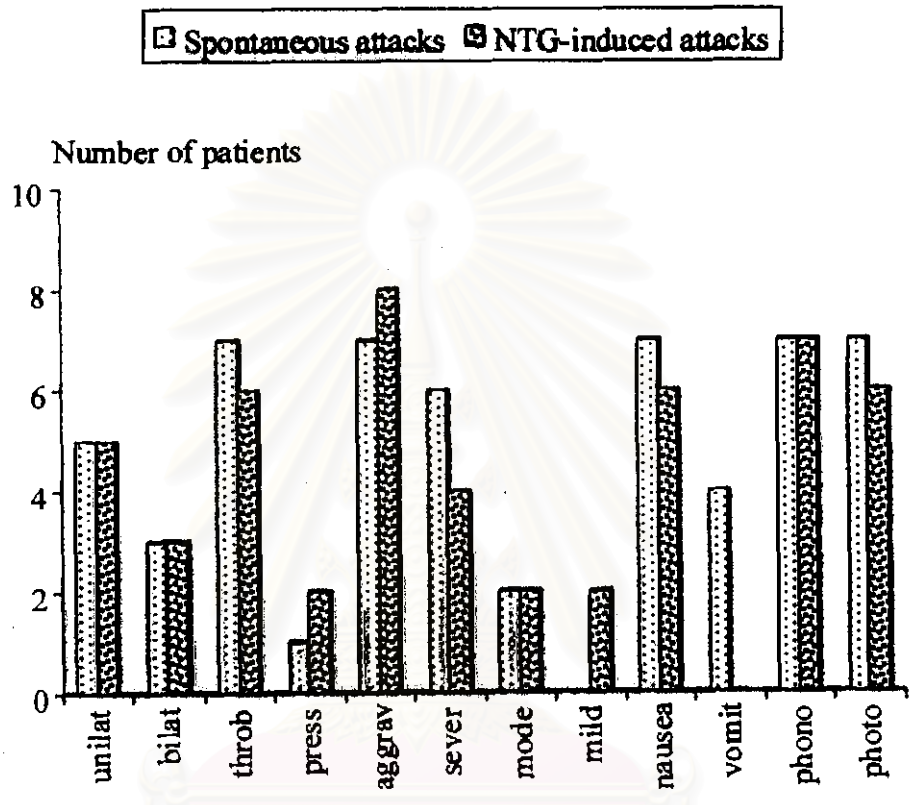

Figure 2.11. Clinical characteristics of NTG-induced migraine. A comparison between spontaneous and NTG-induced migraine in 8 out of 10 migraine patients who developed migraine after NTG infusion (0.5 μg/kg/min for 20 min). (From Thomsen et al., 1994).

NO a Final Common Pathway for Several Migraine Triggers

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 nitrite level in the blood serum from migraine with and without aura. They found a significant increase in cGMP and nitrite 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-HT_{2C} (former called 5-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-HT_{2C} receptor. The vascular response to 5-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-HT_{2C} receptor and, therefore, is likely to cause vascular headache via NO synthesis (Fozard an Kalkman, 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. 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) spreading depression of Leao (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).

Mechanisms of NO Induced Migraine

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., 1993a) 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 causes migraine with a delay of up to several hours (Thomsen et al., 1994). 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, which 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.

REVIEW OF SEROTONIN (5-HT) AND MIGRAINE

INTRODUCTION

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 2.12. 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 neuron (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.

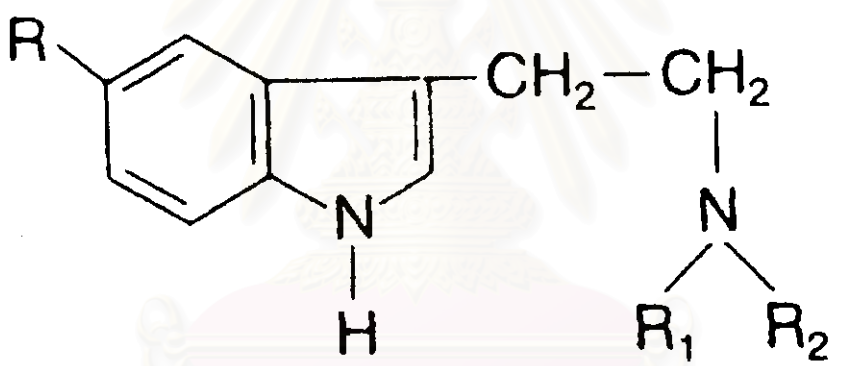

Figure 2.12. Chemical structure of 5-hydroxytryptamine (5-HT)

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 2.13. 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-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 (5-$HT_{1B/D}$) 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, 1993a).

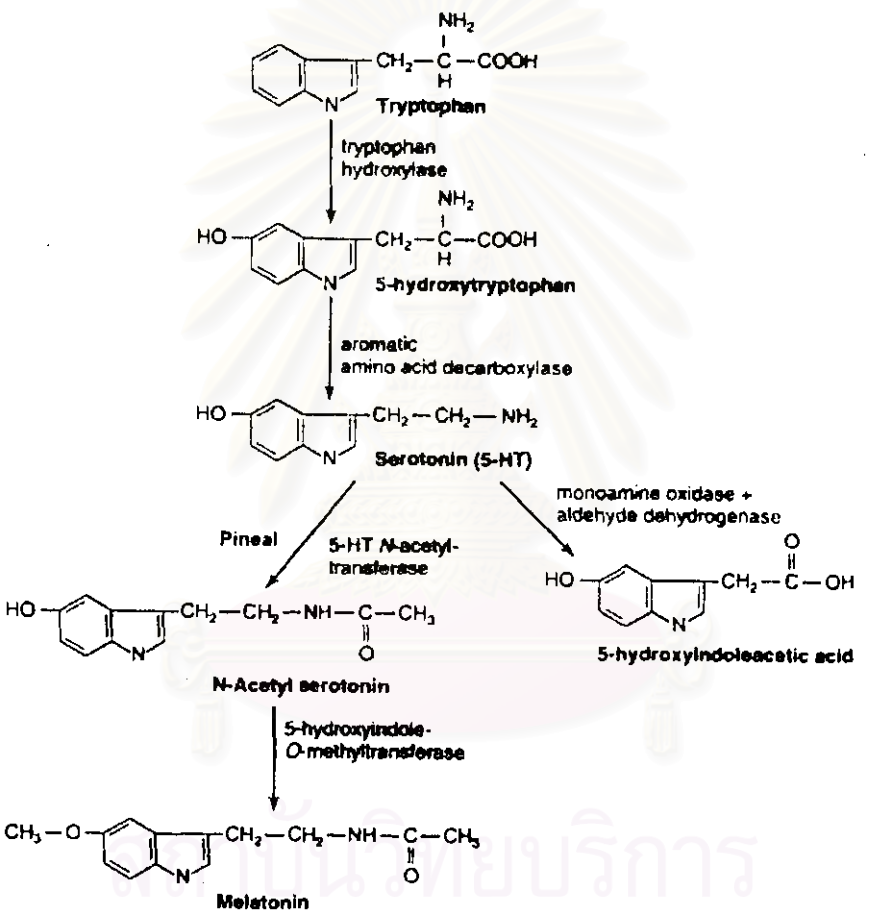

Figure 2.13. The biosynthesis and catabolism of serotonin.

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 2.4) (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 2.5). 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-$HT_{1\text{-like}}$ and were defined by their susceptibility to antagonism to methiothepin, resistance to 5-HT_2 antagonists and potent agonism 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 2.6). The Serotonin Club has recently proposed a new nomenclature for 5-HT receptors based on their operational, structural and transductional properties (Table 2.6). (Humphrey et al., 1993)

Table 2.4. 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 2.5 : 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	5-HT_{1A}	5-HT_{2A}	5-HT_3	5-HT_4	5-HT_{5A}	5-HT_6	5-HT_7	
Serotonin	5-HT_{1B}	5-HT_{2B}			5-HT_{5B}			
Club	5-HT_{1D}	5-HT_{2C}						
	5-HT_{1E}							
	5-HT_{1F}							
Transducer	↓cAMP	$\uparrow P_1$	ion channel	↑cAMP	?	↑cAMP	↑cAMP	

Table 2.6. Classification of Serotonin Receptors

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

$5\text{-}HT_2$
- G protein linked
- Phosphatidylhydrolysis
  - $5\text{-}HT_{2A}$
  - $5\text{-}HT_{2B}$
  - $5\text{-}HT_{2C}$

$5\text{-}HT_3$
- linked to ion channel

$5\text{-}HT_4$
- G protein linked
- Stimulates adenylate cyclase

$5\text{-}HT_5$
- G protein linked
  - $5\text{-}HT_{5A}$
  - $5\text{-}HT_{5B}$

$5\text{-}HT_6$
- G protein linked
- Stimulates adenylate cyclase

$5\text{-}HT_7$
- 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 agonism by 5-CT is no longer required to define 5-HT_1 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 $5HT_2$ 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 5HT_{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: $5HT_{5A}$ 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)

Serotonergic Neuron and Receptor Distribution

There are two morphologically distinct classes of 5-HT axon terminals: fine axons (small varicose axons) and beaded axons (large varicose axons). In the rat, fine terminals come from the dorsal raphe, while beaded terminals originate from the median raphe. Fine 5-HT axons are widely distributed in cerebral cortex, while beaded axons are restricted in distribution to the outer layer of neocortex and selected areas of the limbic system. Beaded 5-HT axon terminals often form baskets or pericellular arrays which surround GABA-ergic inhibitory interneurons. The fine serotonergic axon terminals are selectively damaged by amphetamine, which acts as a neurotoxin, while beaded 5-HT axon terminals are preferentially spared. Thus there are two anatomically and functionally distinct ascending serotonergic systems in the forebrain, with separate origins, distribution, and sensitivity to neurotoxins. The descending 5-HT neurons are biochemically distinct from the rostral system in having peptides [SP, thyrotropin releasing hormone (TRH)] co-stored with 5-HT. Each system may be associated with distinct receptor sites (Boadle-Biber, 1993).

Location of 5-HT Receptors. Two techniques were used to localize receptors for 5-HT namely, radioligand binding and autoradiography. These techniques using nucleic acid probes to detect presence of receptor mRNA.

5-HT_{1A} receptors are widely distributed throughout the brain, with enrichment in the limbic area, the cortex, and certain brainstem nuclei. The highest density is found in the hippocampal formation. Very high densities are found over the raphe nuclei. Basal ganglia, hypothalamus,

and the basal and lateral nuclei of the amygdala show low levels. (Hoyer et al., 1994)

5-HT_{1B} receptors are expressed most abundantly in the striatum and in the purkinje cells of the cerebellum (Maroteaux et al., 1992)

5-HT_{1C} (5-HT_{2C}) receptors are located in high concentrations in the choroid plexus. In addition, they are found in the cortex, basal ganglia, hippocampus, hypothalamus, and midbrain. The thalamus, amygdala, pons-medulla, and spinal cord are poor in 5-HT_{1C} sites. (Molineaux et al., 1989)

5-HT_{1D} most of the 5-HT_1 binding sites in the brain are 5-HT_{1D}. They are found in highest density in the basal ganglia but are widely distributed throughout the brain, being found in the cortex, hippocampus, dorsal raphe, and spinal cord, especially the substantia gelatinosa. (Hoyer et al., 1992)

5-HT_{1E} mRNA is found in the raphe. 5-CT insensitive 5-$HT_{1\text{-like}}$ receptors (5-$HT_{1E/1F}$) are found by binding studies in the caudate, putamen, and olfactory bulbs of rodents (Beer et al., 1993).

5-HT_{1F} m-RNA is found in layer V of the cerebral cortex, the hippocampal pyramidal cells several thalamic nuclei, and the dorsal raphe (Adham et al., 1993).

5-HT_2 The cerebral cortex has the highest density of 5-HT_2 receptors. They are also found in the amygdala, claustrum, hypothalamus, and to a lesser extent the basal ganglia. The thalamus has few receptors. The hippocampus shows spotty labeling. The cerebellum,

brainstem, and spinal cord show low density labeling. (Hoyer et al., 1992)

5-HT_{2F} (5-HT_{2B}) receptor localization has not been reported.

5-HT_3 receptors are present in the cortex, the limbic system, the striatum, the dorsal motor nucleus of the vagus (particularly the solitary tract), the area postrema, the spinal trigeminal nuclei, and the whole length of the spinal cord in the substantia gelatinosa. (Hoyer et al., 1994)

5-HT_4 has not yet been cloned.

5-HT_5 - 5-HT_{5B} mRNA is found in the hippocampus, habenula, and dorsal raphe. (Matthes et al., 1992)

5-HT_6 mRNA is expressed in the corpus striatum, amygdala, cerebral cortex, and olfactory tubercle (Hoyer et al., 1994)

5-HT_7 mRNA is most abundant in the hypothalamus, less abundant in the hippocampus and mesencephalon, and least abundant in the cerebral cortex, olfactory bulb, and tubercle (Hoyer et al., 1994).

5-HT receptors are located both presynaptically (auto-and heteroreceptors) and postsynaptically. The body and dendrites of serotonergic neurons have somatodendritic 5-HT_{1A} autoreceptors which, when activated, inhibit neuronal activity. 5-HT_{1B} and 5-HT_{1D} receptors are located on the terminals of both serotonergic (autoreceptor) and non-serotonergic (heteroreceptor) neurons. Activation of this terminal receptor blocks transmitter release. The 5-HT_{1E} and 5-HT_{1F} receptors may also function as autoreceptors. The 5-HT transporter, located on the

nerve terminal, is responsible for reuptake and termination of the action of 5-HT.(Boadle-Bier, 1993) Activation of the somatodendritic 5-HT_{1A} autoreceptor reduces the neuronal firing rate and 5-HT synthesis. Stimulating the 5-$HT_{1D/B}$ terminal receptor inhibits 5-HT synthesis and release without changing the neuronal firing rate (Boadle-Biber, 1993).

There is a relation between neuronal location, type and receptor subtype. The raphe has two different projections: the dorsal raphe projects to the cortex and the amygdala, where the predominant postsynaptic receptor subtypes are 5-HT_2, 5-HT_{1C}, and 5-HT_3, and probably other new receptors, including 5-HT_6. The lower part of the raphe, the median raphe nucleus, projects to the hippocampus and limbic system, where the main postsynaptic receptors are 5-HT_{1A} and others, including the 5-HT_7 (Boadle-Biber, 1993). Drugs such as buspirone may act in the hippocampus through the 5-HT_{1A} receptor. The location of the receptor and its coupling to the second messenger may determine whether a mixed agonist/antagonist drug acts more as an agonist or an antagonist, as discussed above (Boadle-Biber, 1993).

5-HT AND MIGRAINE

During a migraine attack, platelet 5-HT decreases, urinary 5-HIAA increases in some patients. These changes in 5-HT are probably epiphenomena, since changes in the plasma 5-HT levels are probably not of clinical significance in regulating cerebral arterial tone. Other evidence suggesting a role for 5-HT is based on the observation that headache can be precipitated by reserpine, relieved by 5-HT or 5-HT_1 agonists, and blocked by treatment with methysergide (Ferari et al., 1989).

Urinary 5-HIAA increases in about 50% of migraine patients during an attack, and platelet 5-HT content falls rapidly by 20-40% at the onset of the attack. Platelet 5-HT represents less than 10% of the total body 5-HT store (Ferari and Saxena, 1993). Of the daily total 5-HIAA secretion (3-12 mg), the contribution from the platelet is less than 0.1-0.2 mg, corresponding to the release when platelets are destroyed. There is a considerable variation in 5-HIAA excretion during headache-free periods. In most studies some, but not all, migraineurs show increased urinary 5-HIAA excretion during attacks. The amount from the platelets (0.2 mg) would account for only a minor amount of the increase in 5-HIAA. Some groups have found a decrease in urinary 5-HIAA. The difference in methodology of the studies may account for these differences. If there is a disturbance in 5-HT metabolism it is a general phenomenon not limited to the platelets (Ferari and Saxena, 1993).

Between 70 to 100 per cent of patients in earlier series showed an average fall of platelet 5-HT of about 34 per cent. Ferrari et al., (1989) found an ictal decrease in patients with migraine without aura (16/20) but only in 2/10 patients with migraine with aura. During attacks they found an increase in plasma 5-HT levels which they interpreted as being due to decreased 5-HT degradation.

Headaches which may be similar to migraine can be triggered by 5-HT releasing agents such as fenfluramine or reserpine and exacerbated by selective inhibition of 5-HT reuptake by drugs such as zimelodine (Fozard, 1982). A specific compound, mCPP, can trigger migraine, conceivably by activation of the 5-HT_{1C} (5-HT_{2C}) receptor (Fozard, 1989). 5-HT infusion has been said to abort a migraine attack but this could not be replicated by Sommerville in 1976. 5-HT_1 agonists such as

ergotamine, dihydroergotamine (DHE), and sumatriptan can abort a migraine attack. However, these finding can not be directly view as induce for 5-HT deficiency, their effectiveness can be related to agonism of 5-HT_1 inhibitory heteroreceptors on the trigeminal nerve blocking neurogenic inflammation and pain transmission (Goadsby and Hoskin, 1996; Martin et al., 1997). In addition, DHE has been shown to bind to and inhibit the activity of the nucleus caudalis of the trigeminal complex in the brainstem (Goadsby et al., 1991).

Some believe that abortive migraine drugs are agonists at the 5-HT_{1D} and/or 5-HT_{1B} receptor and that preventive drugs are antagonists of 5-HT_2, or 5-HT_{1C} (5-HT_{2C}) receptors, or modulate serotonergic neurons (Peroutka, 1990).

5-HT_1 receptor and migraine. Ergotamine, DHE, sumatriptan and zolmitriptan show high affinity for the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors, suggesting that these receptors are involved in the action of the abortive migraine drugs (Pascual, 1996; Martin, 1997; Goadsby et al., 1998).

5-HT_{1F} receptor and migraine. The 5-HT_{1F} receptor has a high affinity for the anti-migraine drug sumatriptan, suggesting that part of this drug's action may be mediated by this receptor (Fozard, 1992).

5-HT_2 receptor and migraine. The classic 5-HT antagonist prophylactic drugs are pizotifen, cyproheptadine, and methysergide, but there is no correlation between their 5-HT_2 receptor affinity and their clinical effectiveness. This suggests that 5-HT_2 receptor binding may be unrelated to the effectiveness of the drug. Some 5-HT_2 receptor antagonists are ineffective in migraine. Since these drugs are 5-HT_2

receptor antagonists and are of no value in migraine prophylaxis, the unifying hypothesis that migraine prophylactic drugs are all 5-HT_2 receptor antagonists is invalid (Fozard, 1982).

The observation that 5-HT_2 agonists can act as pronociceptive agents has not been consistently confirmed. It is possible that 5-HT has opposite functions depending on the part of the pain pathway being considered; 5-HT may well participate in the detection of nociceptive events and also in the converse, leading to hypoalgesia (Silberstein, 1994).

5-HT_{2C} (5-HT_{1C}) receptor and migraine. The antiserotonin prophylactic drugs are potent antagonists at the 5-HT_{2C} (5-HT_{1C}) receptor while m-CPP, a 5-HT_{2C} (5-HT_{1C}) receptor agonist induces migraine in susceptible individuals (Panconesi and Sicuteri, 1997).

In 1988, Brewerton et al. found that m-CPP, a major metabolite of the antidepressant trazadone, induces migraine hours after the immediate pharmacologic response to the drug (monitored by elevation of plasma cortisol and prolactin) is over, in subjects with family history of migraine or prior migraine. Gordon et al., (1993) using a lower dose of oral m-CPP (0.25 mg/kg) than Brewerton (1988) and found a similar delay in headache onset. However, Gordon et al., (1993) found that m-CPP induced headache in 5 of 8 migraineurs and in 4 of 10 normal controls (non-migraineurs). During the delay prior to the onset of head pain, subjects experience the prodrome of migraine: cognitive difficulties or feelings of exhilaration, exhaustion, anxiety, or depression. Thus m-CPP induces not the headache of migraine, but the prodrome. The headache begins hours after the prodrome (Silberstein et al., 1992). Abortive

migraine drugs, ergotamine and DHE, are less potent 5-HT_{1C} (5-HT_{2C}) receptor agonists (approximately 100-fold less potent). Coexistent 5-HT_{1D} agonism may block 5-HT_{1C} (5-HT_{2C}) activation. Methysergide, cyproheptadine, and pizotifen, effective migraine prophylactic drugs, are 5-HT_{1C} (5-HT_{2C}) receptor antagonists again suggesting involvement of the 5-HT_{1C} (5-HT_{2C}) receptor. Ketanserin, a selective 5-HT_2 and a poor 5-HT_{1C} (5-HT_{2C}) receptor antagonist, is not effective in migraine prophylaxis. Sergolexol, which is a 5-HT_{1C} (5-HT_{2C}) receptor antagonist, is ineffective in migraine; this casts doubt on the unifying concept that it is the 5-HT_{1C} (5-HT_{2C}) receptor antagonist that is responsible for migraine prophylaxis (Fozard, 1989).

5-HT_3 Receptor and Migraine. The excitatory 5-HT_3 receptors are widely distributed in both autonomic and sensory nerves. 5-HT-induced pain and hyperalgesia in the periphery is blocked by selective 5-HT_3 antagonists, suggesting mediation by the 5-HT_3 receptor. 5-HT_3 receptors are concentrated in the CNS areas involved in pain processing and integration of the emetic reflex, such as the nuclei of the trigeminal and vagus nerves, nucleus tractus solitarius, the substantia gelatinosa and the area postrema. However, clinical studies of 5-HT_3 receptor agonists have not yet shown them to be effective in migraine prophylaxis (Fozard, 1990).

5-HT_6 and 5-HT_7 Receptors and Migraine. The 5-HT_6 and 5-HT_7 excitatory receptors are linked to the production of cAMP. They are highly expressed in the limbic system and neocortex and have potent interactions with the tricyclic antidepressants, the atypical antipsychotic clozapine, ergotamines, and methiothepin. The efficacy of these drugs may be explained by their interaction with these receptors (Shen et al., 1993).

REVIEW OF EXPERIMENTAL HEADACHE

The mechanisms of spontaneous migraine attacks are difficult to study because patients must be transported to the laboratory during attacks, when they have pain and nausea. During the migraine attack, changes in cerebral blood flow and in the blood velocity of large arteries have been demonstrated (Olesen et al., 1990; Friberg et al., 1991). These changes are dynamic. Therefore, it is crucial that the time factor be considered and that changes be related to the appropriate phase in the migraine attack. A model of experimental headache may overcome these limitations. According to clinical experience, migraine attacks may be precipitated by many factors such as food, red wine, light and stress, but during controlled conditions these agents often fail to induce migraine (Shiffman et al., 1987).

Therefore, animal models have been developed in order to investigate the pathogenesis of migraine. Several models have been used in recent years. (Table 2.7) The end result of such a model being to define more precisely the anatomy, physiology and pharmacology of this disease.

The structures stimulated in models of migraine are represented in Table 2.8. To achieve a reasonable model of migraine it is essential to activate a structure or structures that may be expected to be involved in the process. Since migraine is generally agreed to involve trigeminally-innervated structures that should be dural or intracranial large vessels. The dural/pial arteries were chosen since they are painful in humans when stimulated. Stimulation of the superior sagittal sinus results in neuropeptide release similar to what is seen in humans (Goadsby et al.,

1990). Moreover, anti-migraine drugs, such as sumatriptan, block the effects of trigeminal activation just as they are in humans after successful treatment of acute attacks of both migraine and cluster headache.

Table 2.7. Current animal models of migraine

Site	Measurement
Trigeminal ganglion stimulation	cerebral blood flow plasma protein extravasation
Meningeal irritation blood other chemical	Fos
Cranial vessel stimulation	neuropeptides Fos cerebral blood flow trigeminal nucleus recording thalamic neuron recordings
Cranial and other vascular dynamics	arteriovenous anastomoses Saphenous vein studies

Table 2.8. Structures stimulated in models of migraine

Site	
Blood vessels/ dura mater	
	superior sagittal sinus
	middle meningeal artery
	direct dural stimulation
	extracranial vessel stimulation
Trigeminal ganglion	
Greater occipital nerve	
Stimulation means	
	electrical
	mechanical
	chemical

Two Main Experimental Models of Headache in Animal.

Animal models have been developed to investigate migraine pathogenesis. The first model, the trigeminal stimulation model, is a quantitative measure of neurogenic inflammation within the dura mater caused by chemical or electrical stimulation of trigeminovascular fibers. The second model measures the expression of the immediate early gene c-*fos* within the trigeminal nucleus caudalis after noxious chemical stimulation of the meninges.

Model 1: Trigeminovascular Extravasation – A Model of Neurogenic Inflammation

Electrical stimulation of the trigeminal ganglion or intravenous injection of the irritant capsaicin activates small-caliber C fibers and releases neuropeptides from nerve terminals in the meninges of rodent. With stimulation, neuropeptide (i.e. CGRP) levels increase in draining venous effluent (Buzzi et al., 1991; Kurosawa et al., 1995). Once, they released, the neuropeptides initiate a cascade of events known collectively as neurogenic inflammation, which includes (1) an increase in leakage of plasma from meningeal vessels; (2) vasodilatation; (3) the formation of endothelial microvilli, endothelial vesicles, and vacuoles, specifically within postcapillary venules (Dimitriadou et al., 1992) (4) activation and degranulation of mast cells (Dimitriadou et al., 1991), and (5) platelet aggregation.

The leakage of radiolabeled albumin from meningeal blood vessels is used to estimate neurogenic inflammation. Electrical stimulation of the trigeminal ganglion on one side is preceded or followed by a femoral vein

injection of ^{125}I-albumin. Ten minutes later, the animals are perfused to remove residual labeled albumin from the intravascular compartment, and the dural tissues from both the stimulated and unstimulated sides are harvested. The residual radioactivity from extravasated plasma on the stimulated side is then compared with that on the unstimulated side, and the comparison is expressed as a ratio. The effect of a test drug on this ratio is assessed.

Model 2: C-*fos* Expression in Trigeminal Nucleus Caudalis

The second model of headache measures the expression of c-*fos*, an immediate early gene, as a semiquantitative marker of cephalic head pain. Expression of c-*fos* is widely used as a nonspecific indicator of neuronal activation (Morgan and Curran, 1991) and can be induced in the brain by a variety of stimuli (Morgan and Curran, 1986; Morgan et al., 1987). C-*fos*-like immunoreactivity (c-*fos*-LI) within the dorsal horn of the spinal cord (lamina I,II$_o$) is a well-characterized marker of painful peripheral stimulation. Noxious somatic, visceral, and articular stimulation results in an increase in c-*fos*-LI in lamina I,II$_o$ of rat dorsal horn (Menetrey et al., 1989). Morphine pretreatment reduced formalin injection-evoked c-*fos* expression within the dorsal horn in a dose-dependent and naloxone-reversible manner (Presley et al., 1990). c-*fos*-LI within lumbar spinal cord after hind-paw formalin injection correlates with the results of behavioral testing (Goadsby et al., 1991).

C-*fos*-LI can be evoked within lamina I,II$_o$ of the TNC (an area analogous to the dorsal horn in the spinal cord) by stimulating meningeal nociceptive neurons using intracisternal irritants. A small catheter is placed through the atlanto-occipital membrane into the cisterna magna of

anesthetized rats or guinea pigs. Five and one-half hours later, either drug or vehicle is administered (intravenously or intraperitoneally). Six hours after catheter placement, a chemical stimulus (either autologous blood or a dilute capsaicin solution) is injected into the cisterna magna. Two hours later (at the peak of c-*fos* expression), animals received anesthetized overdosed and perfused via the ascending aorta. The brainstems and attached spinal cords are the dissected, sectioned (50 μm), and processed immunohistochemically. The cells exhibiting c-*fos*-LI are the counted in every third 50-μm section at three sampling levels within the TNC (Cutrer et al., 1995b).

Previously, Nozaki et al. (1992) demonstrated that intracisternal injection of autologous blood would induce c-*fos* expression within lamina I,II_o of the TNC. The number of cell expression c-*fos* corresponded to the amount of injectate and was reduced after destruction of unmyelinated fibers by neonatal capsaicin treatment or by surgical trigeminal transection.

Several compounds known to block neurogenic inflammation and migraine headaches also attenuate c-*fos*-LI selectively within the TNC, pretreatment with sumatriptan, dihydroergotamine, or morphine significantly decreased c-*fos* expression in TNC after intracisternal blood or carrageenan injection (Nozaki et al., 1992). CP-122,288 (a sumatriptan analogue that potently blocks neurogenic inflammation) attenuates c-*fos* expression in the TNC at very low doses ($\geq$ 100 pmol per kg) after intracisternal capsaicin (Cutrer et al., 1995c). The nonpeptide NK1-receptor antagonist RPR 100,893 also attenuates c-*fos*-LI in the TNC at doses of greater than or equal to 1 μg per kg while its enantiomer, RPR 103,253 (100 μg per kg), is inactive (Cutrer et al., 1995b).

An Experimental Human Headache Model.

The need for valid experimental human models of headache is obvious. According to clinical experience migraine attacks may be provoked by many factors, but during controlled conditions these agents often fail to induce migraine or the use is limited by severe side effects (Iversen and Olesen, 1993).. A suitable headache-inducing compound should be safe, well tolerated, easy to control, and able to induce migraine headache in migraineurs. Intravenous nitroglycerin fulfills these demands. It is an old and well-tolerated drug and has a very short half-life. It easy crosses biologic membranes, including the blood brain barrier. The side effects are a feeling of warmth (but no blushing, which could disturb investigator blindness), nasal congestion, and pain/pressure behind the eyes. Hypotension seldom occurs when subjects are supine during infusion. NTG is denitrated to NO and vasoactive S-nitrosothiols. Both activate sGC resulting in increased intracellular cGMP , an increase in cytosolic and vasodilatation (Ignarro et al., 1981). NTG may be regarded as an exogenous source of NO. Endogenous NO is the main endothelium-derived relaxing factor (EDRF) and is involved in numerous other physiological process including intracranial vasomotor activity (Moncada et al., 1991).

Basic Characteristics of the NTG Model

The dose-response relationship between headache and NTG was investigated in 10 healthy subjects with no history of migraine (Iversen et al., 1989b). NTG was infused in periods of 10 minutes separated by wash-out periods. Doses were increased successively from 0.25, 0.5, 1.0 and 2.0 μm/kg/min. The washed-out periods varied from 10 to 30

minutes to ensure that the headache had disappeared or was very mild before the next NTG infusion. One placebo infusion, and wash-out period was randomly inserted double blind. Headache was scored on a numerical verbal rating scale from 0 to 10; 1 represent a very mild headache (including feeling of pressing or pulsating), 5 a moderate headache, and 10 the worst possible headache. To estimate headache reproducibility, a retest was performed after at least 1 week. Headache developed in 9 of 10 subjects. The one subject who did not experience headache was the same on both study days. The NTG-induced headache intensity was mild or moderate. Maximal headache score occurred within 2-5 min and declined rapidly after termination of the NTG infusion. A reproducible ceiling effect in headache score at 0.5 μg/kg/min was found from the dose response curves (Figure 2.14). The day-to-day variation in headache intensity was acceptable. On each of the two study days, five infusions were administered to each of the 10 subjects. A total of 50 paired infusions could thus be compared. Twenty-five did not differ in maximal headache response. 20 differed by 1 headache score (out of 10 possible), 4 differed by 2, and only 1 differed by 3 headache scores from day-to-day. Also the characteristics of the induced headaches were reproducible (Figure 2.14). In all subjects the headache was bilateral on both study days. The headache quality was pulsating in seven subjects, pressing in two and did not vary between days. A feeling of warmth was reported in five subjects at the highest doses but lasted only a few minutes. No accompanying flushing (such as seen with histamine) was observed and therefore the investigator was not unblinded.

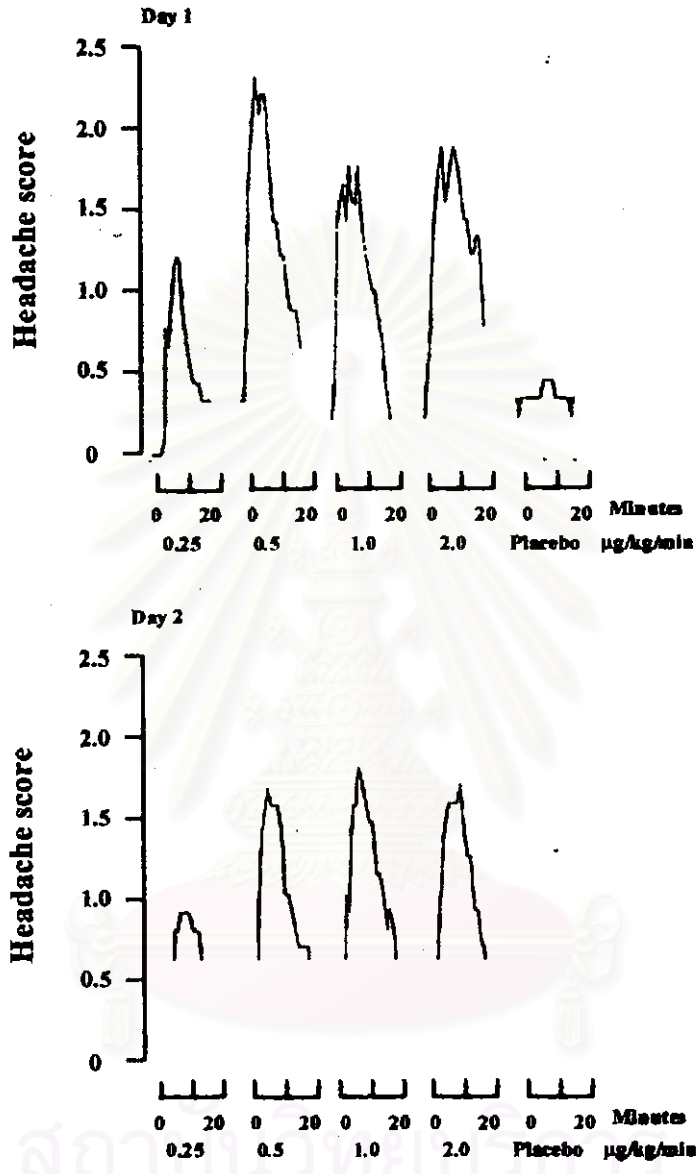

Figure 2.14. Average headache scores, during NTG infusion and wash-out periods, day 1 (A) and day 2 (B) (n=9). The NTG infusion were discontinuued at 10 minutes. (From Iversen and Thomsen, 1989b)

During NTG infusions both headache and diameter increased rapidly, stabilized after 20 min and did not vary significantly throughout the rest of the infusion period. Although headache intensity changed over time in the individual subjects, tolerance was not seen either in the headache or in the dilatory response (Iversen et al., 1993).

NTG-headache and Hemodynamics Related to Changes During Migraine Headache

In contrast to migraine headache, NTG headache is usually bilateral and mild to moderate in intensity. In both headaches the pain quality is pulsating and the intensity is aggravated by routine physical activity. In accordance with the criteria for migraine without aura, it is only necessary to fulfill two of the following four pain criteria: moderate or severe intensity, unilateral location, pulsating quality and aggravation by physical activity. NTG headache thus fulfills the pain criteria for migraine. However, requirements for accompanying symptoms (photo and phonophobia, nausea and vomiting) are rarely fulfilled in NTG headache in non-migraineurs.

During migraine headache, both the temporal artery and the middle cerebral artery are dilated on the headache side compared to the non-headache side (Friberg et al., 1991; Iversen et al., 1990). The observed changes in rCBF during the aura phase show no time relationship to changes in headache (Olesen et al., 1990), and the abnormalities in rCBF have never been observed in migraine patients without aura (Olesen et al., 1990). Changes in CBV during migraine headache are completely unknown. Thus, the hemodynamic responses during NTG headache and migraine without aura headache are very similar, with unchanged rCBF and affection of both intra- and extracranial large arteries.

NITROGLYCERIN (GLYCERYL TRINITRATE)

History: NTG, an organic nitrate, has been used clinically for many years as vasodilator. It was first synthesized in 1846 by Sobrero, who observed that a small quantity of the oily substance placed on the tongue elicited a severe headache. The chemical structure was illustrated in Figure 2.15. Constantin Hering, in 1847, developed the sublingual dosage form. Subsequently, William Murrell (1848) established the use of sublingual NTG for relief of the acute anginal attack and as a prophylactic agent to be taken prior to exertion. The empirical observation that organic nitrates could be used safely for the rapid, dramatic alleviation of the symptoms of angina pectoris led to their widespread acceptance by the medical profession.

Chemistry: NTG is polyol esters of nitric acid. Nitrate esters is characterized by a sequence of carbon-oxygen-nitrogen, whereas nitro compound possess carbon-nitrogen bond ($C\text{-}NO_2$). Thus, glyceryl trinitrate (GTN) is not a nitro compound, and it is erroneously called nitroglycerin; however, this nomenclature is both widespread and official. NTG is moderately volatile, oily liquids. The fully nitrated polyols are lipid soluble, whereas their incompletely nitrated metabolites are more soluble in water. In the pure form (without an inert carrier such as lactose), nitroglycerin is explosive. The organic nitrates and nitrites and several other compounds that are capable of denitration to release NO have been collectively termed *nitrovasodilators*. NO activates guanylyl cyclase, increasing intracellular levels of cGMP, and thereby produces vasodilatation (Thadani, 1992).

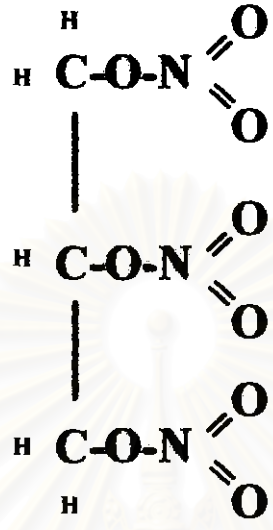

Figure 2.15. Chemical structure of nitroglycerin (NTG).

Mechanism of Action of NTG

NTG is one of several available organic nitrate esters used clinically as vasodilators to treat patients with angina, congestive heart failure, and other cardiovascular disorders. NTG has been used in this capacity for more than a century, although its mechanism of action remained a complete mystery until the 1970s. Other organic nitrate esters that possess a similar mechanism of action include isosorbide dinitrate, pentaerythriol tetranitrate, and erythrityl tetranitrate. Organic nitrite esters (iso amyl nitrite) and sodium nitroprusside, although chemically distinct from NTG, have a similar mechanism of action.

Release of NO

Inorganic nitrite (NO_2^-) reacts in aqueous acid to yield nitrous acid and nitric acid, but this reaction is slow at biologic pH, and nitrite is therefore only a weak vasodilator. In the presence of free thiols or sulfhydryl groups, however, nitrite rapidly generates NO by forming the labile intermediate S-nitrosothiol (Ignarro et al., 1981). For example, nitrite reacts with cysteine to yield S-nitrosocysteine, which in turn, spontaneously decomposes to generate free NO plus cystine (the disulfide of cysteine). S-Nitrosothiols are potent vasodilators that mimic the cardiovascular hemodynamic actions of NTG (Ignarro et al., 1981). Although NO activates sGC in the absence of added thiols, enzyme activation by nitroglycerin and other organic nitrate esters cannot occur appreciably in the absence of added cysteine (Ignaro et al., 1981). The active species is NO that is generated spontaneously from the labile S-nitrosocysteine formed in the reaction. Enzymatic activation by S-nitrosocysteine is indistinguishable in mechanism from that caused by NO. These findings are compatible with earlier studies showing the requirement of tissue thiols for arterial relaxation elicited by organic nitrate esters and suggesting that NTG interacts with sulfhydryl-containing receptors (Needleman et al., 1973). The mechanism of action for NTG is shown schematically in Figure 2.16.

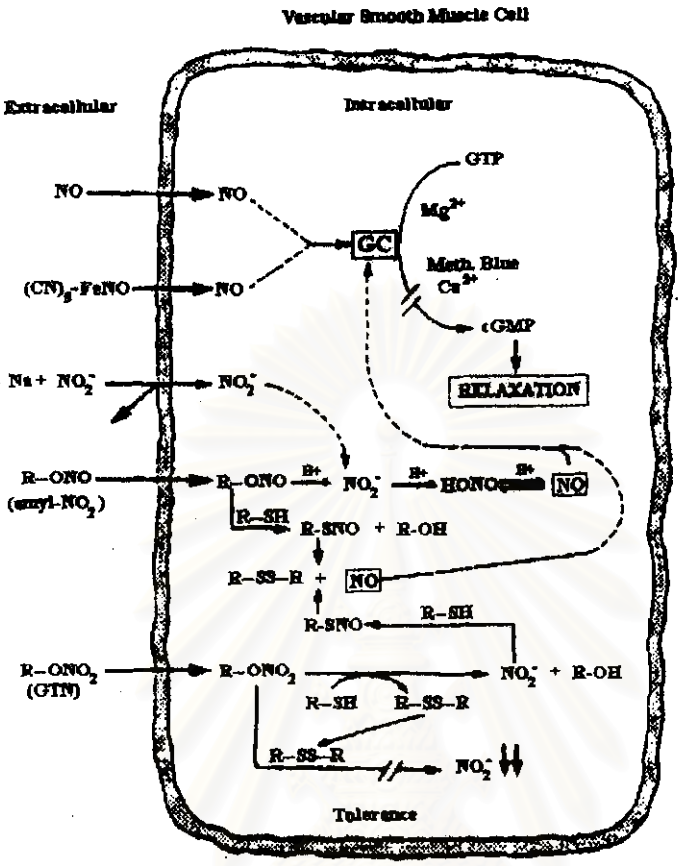

Figure 2.16. The schematic illustration shows the purposed mechanism of vascular smooth muscle relaxation elicited by nitrovasodilators. NO is lipophilic, readily permeates the plasma membrane, activate cytoplasmic GC, and elevates intracellular cGMP. Inorganic nitrite (NO_2^-) is charged, and only limited amount can permeate to cell, but intracellular nitrite can be convert to NO. Lipophilic organic nitrite, or nitrate esters (R-OH), readily permeate cells and can be convert to nitrite and NO in chemical reactions that are facilitated by thiols (R-SH) and acidic condition. S-nitrosothiols (R-SNO) are labile intermediates that decompose spontaneously with the generation on NO. Organic nitrate esters ($R-ONO_2$) specifically need one pool of thiols, such as cysteine, to generate NO, and intracellular thiols deficiency results in the development of selective tolerance or refractoriness to their pharmacologic actions. (Ignarro et al., 1981). (CN)5-FeNO=nitroprusside, GTN=Glyceryl trinitrate or nitroglycerin, GPT=guanosine triphosphate, HONO=nitrous acid, Meth. Blue=methylene blue, R-ONO=organic nitrite ester, R-SS-R=disulfide.

Tolerance

The repeated or long-term administration of high doses of NTG can lead to the development of tolerance or refractoriness to the vascular smooth muscle relaxing effect of NTG and other organic nitrate esters with little or no cross-tolerance to sodium nitroprusside. The first clue to the mechanism of the development of tolerance to NTG came from studies that also provided the first clue to its mechanism of vascular smooth muscle relaxation (Needleman et al., 1973). Tissue sulfhydryl groups were found to be needed for arterial relaxation elicited by organic nitrate esters, sodium nitroprusside, inorganic nitrite, and related vasodilators. The relaxant effects of all agents were blocked by nonselective thiol alkylating agents. On repeated testing, refractoriness to vascular smooth muscle relaxation developed only to the organic nitrate esters. The development of tolerance to NTG was associated with a concomitant decrease in detectable free sulfhydryl groups as well as the formation of nitrite from NTG in the tissue. Normal tissue responsiveness was restored by adding thiol reducing agents. The conclusion reached was that NTG interacts selectively with a population of tissue sulfhydryl groups that is distinct from those sulfhydryl groups that interact with nitroprusside or nitrite. A plausible hypothesis was that NTG oxidizes selective sulfdryl-containing receptors to the disulfide form and that the latter displays only a low binding affinity for NTG. More recent studies were confirmatory and showed that the development of tolerance to vascular smooth muscle relaxation elicited by NTG is accompanied by a concomitant development of tolerance to the cGMP accumulating action of NTG (Keith et al., 1982). Vascular tolerance to NTG was not associated with appreciable cross-tolerance to nitroprusside or cGMP.

Absorption, Fate and Excretion.

The biotransformation of NTG is result of reductive hydrolysis catalyzed by the hepatic enzyme glutathione-organic nitrate reductase. The enzyme converts the lipid-soluble organic nitrate esters into more water-soluble denitrated metabolites and inorganic nitrite. The partially denitrated metabolites are considerably less potent vasodilators than are the parent compounds. However, under certain conditions their activity may become important. Since the liver has an enormous capacity to catalyze the reduction of organic nitrates, their biotransformation is a major factor in determining oral bioavailability and duration of action. One molecule of NTG reacts with two molecules of reduced glutathione to release one inorganic nitrite ion from either the 2 or 3 position; the products are 1,3- or 1,2-glyceryl dinitrate and oxidized glutathione.

In human beings, peak concentrations of NTG are found in plasma within 4 minutes of sublingual administration the compound has a half life of 1 to 3 minutes. Dinitrate metabolites, which are about ten times less potent as vasodilators, appear to have a halflife of approximately 40 minutes.

Correlation of Plasma Concentration of Drug and Biological Activity

Intravenous administration of NTG in anesthetized animals produces the same transient (1 to 4 minutes) decrease in blood pressure. Since denitration markedly reduces the activity of the organic nitrates, their rapid clearance from blood indicates that the transient duration of action under these conditions correlates with the concentrations of the parent compounds. The rate of hepatic denitration is characteristic of each nitrate. In addition, it is influenced by hepatic blood flow or the presence of hepatic disease. In experimental animals, injection of

moderate amounts of organic nitrates into the portal vein results in little or no vasodepressor activity, indicating that a substantial fraction of drug can be metabolized during its first circulation through the liver.

Toxicity and Untoward Responses

Untoward responses to the therapeutic use of organic nitrates are almost all secondary to action on the cardiovascular system. Headache is common and can be severe. It usually decreases over a few days if treatment is continued and often can be controlled by decreasing the dose. Transient episodes of dizziness, weakness, and other manifestrations associated with postural hypotension may develop, particularly if the patient is standing immobile, and may occasionally progress to loss of consciousness. This reaction appears to be accentuated by alcohol. It may be seen with very low doses of nitrates in patients with autonomic dysfunction.