CHAPTER II

LITERATURE REVIEWS

Migraine

Migraine is a recurring neurological disorder commonly known as a throbbing unilateral head pain that is readily aggravated by routine physical activities. Clinical definition of migraine includes a host of neurological symptoms other than pain: nausea, photophobia, phonophobia, osmophobia, fatigue, and numerous disturbances in autonomic, mental, sensory, and motor functions [7]. Migraine affects 18% of women and 6% of men, and ranks among the world's most disabling medical illnesses [8].

The pathophysiology of migraine is not fully understood. However, migraine pain is thought to be driven by activation and sensitization of peripheral neurons in the trigeminal ganglion that innervate the meninges (i.e. meningeal nociceptors) as well as the activation and sensitization of central trigeminovascular neurons which are located in the trigeminal nucleus caudalis (TNC) of the brainstem and in C1 and C2 regions of the spinal cord. Several lines of evidence suggest that activation of meningeal nociceptors can be initiated locally by release of inflammatory mediators around meningeal blood vessels [9, 10]. Upon such activation, meningeal nociceptors become hyperresponsive (sensitized) to the otherwise innocuous vascular pulsation, resulting in the characteristic throbbing of migraine pain [11, 12]. The sustained firing of sensitized meningeal nociceptors eventually leads to activation and subsequent sensitization of central trigeminovascular neurons [13]. The central neurons process sensory signals that originate not only from the dura, but also from the periorbital skin. The sensitization of these neurons causes an increased responsiveness not only to mild changes in intracranial pressure but also to innocuous skin stimulation. This central sensitization, which occurs during migraine attack in many patients [14], is manifested as cutaneous allodynia (i.e. enhanced periorbital skin sensitivity).

Inflammatory mediators relevant to migraine pathophysiology

Various mediators including ions, free radicals, the complement system, kinins, and cytokines are important for inflammatory processes in migraine. The mediators that are highly relevant to migraine pathophysiology which being used in the mixture called "inflammatory soup" are reviewed here.

A. Histamine

There are many papers reporting the involvement of histamine in migraine pathogenesis. In patients with migraine history, the plasma histamine level was higher than normal subjects and the histamine level further increased during migraine attack [15]. Histamine release also increased from basophils taken from migraineurs [16]. Mast cell, another cell which can release histamine, was reported to be activated in patient with cluster headache. This evidence indicates the involvement of the dura mast cell activation in migraine headache [17]. Mast cell vasodilatory molecules including histamine could be responsible for the vasodilatory phase of the migraine, associated with throbbing pain. Mast cells are located perivascularly in close proximity to neurons, especially in association with substance P (SP) containing neurons [18]. Histamine application to the nasal mucosa induces release of calcitonin gene-related peptide (CGRP) and SP from peripheral terminals of trigeminal ganglion in guinea pig [19]. Furthermore, it is suggested that histamine may be involved in the control of blood-brain barrier permeability in rodents [20]. It has been proposed that histamine via a H1-receptor stimulates the release of nitric oxide in cerebral arteries which in turn triggers the migraine attacks [21].

B. Serotonin

Serotonin (5-hydroxytryptamine; 5-HT) is a monoamine neurotransmitter that appears to be involved in a wide variety of physiological functions and behaviors, such as eating, sleep, circadian rhythmicity, and neuroendocrine function. Perturbation of the serotonin system by different drugs can elicit alterations in behaviors. Drugs affecting serotonergic neurons and their receptors are used to treat several diseases such as depression, anxiety disorder, and schizophrenia [22].

Serotonin is synthesized in certain brain cells. The initial step of serotonin synthesis is the facilitated transport of amino acid L-tryptophan from blood into brain. The second step is converting tryptophan to 5-hydroxytryptophan by the action of tryptophan hydroxylase. This step appears to be rate-limiting step in serotonin synthesis. Finally, aromatic amino acid decarboxylase metabolizes 5-hydroxytryptophan to 5-hydroxytryptamine. Blood platelets also contain serotonin. However, they accumulate serotonin from plasma by active transport mechanism.

Serotonin exerts its effect on serotonin receptors which are classified into many classes: 5-HT_1 to 5-HT_7 receptors. All of 5-HT receptors, except 5-HT_3 receptor, are member of the G-protein-coupled receptor (GPCR) superfamily. The 5-HT_3 receptor belongs to ligand-gated ion channel superfamily [23]. Specific receptor subtypes have recently been associated with either the pathogenesis or the treatment of migraine headache [24]. A role for serotonin in migraine has been supported by sudden changes in circulating levels of serotonin and its metabolites observed during the early phases of a migraine attack [25], along with the ability of serotonin-releasing agents to induce migraine-like symptoms [26].

At present, it is not clear whether 5-HT receptor subtypes were associated with elicitation of nociceptive hypersensitivity. The previous physiological and pharmacological studies indicated the involvement of 5-HT_{1A}, 5-HT_{2A}, 5-HT_3 and 5-HT_7 receptor subtypes in facilitating peripheral nociceptive transmission. 5-HT_{1A} receptor agonist could produce hyperalgesia in a mechanical test via direct action on C-fiber neurons [27]. The 5-HT_2 receptor family has also been suggested to play a role in the development of headache, as many prophylactic drugs have inhibitory actions at 5-HT_{2C} and 5-HT_{2B} receptors [28]. Activation of 5-HT_{2A} receptor leads to an enhancement of nitric oxide production in trigeminovascular pathway [29]. Activation of 5-HT_3 receptor was shown to stimulate neuropeptide release from the rat spinal cord [30]. 5-HT_7 receptor is thought to be involved in mediating 5-HT-induced hyperalgesia in rat [31]. A role for the 5-HT_7 receptor in the precipitation of migraine headache has recently been proposed on the basis of its vasodilatation role and the binding properties of existing prophylactic drugs.

C. Bradykinin

Bradykinin is 9–11 amino-acid peptide that acts on blood vessels and is involved in cardiovascular regulation, inflammation, and pain. They also stimulate postganglionic sympathetic neurons, degranulate mast cells to release various inflammatory mediators, and cause plasma extravasation by contraction of vascular endothelial cells. They are potent algogenic substances and induce pain by directly stimulating nociceptors and sensitizing them to innocuous stimuli.

Bradykinin exerts a number of pro-inflammatory effects via two distinct GPCR subtypes (B1 and B2), including the release of prostanoids, cytokines, and free radicals from a variety of cells. B2 receptors are constitutively expressed, whereas B1 receptors are inducible and are up-regulated in the presence of cytokines, endotoxins, or tissue injury. These receptors have been defined on the basis of pharmacologic criteria. Bradykinin is endogenous agonists for B2 receptors [32, 33]. B1 receptors have been implicated in the chronic phase of pain and inflammation. In contrast, B2 receptors are important mediators of the acute phase of inflammation (arterial relaxation, venoconstriction, increased permeability) and of somatic and visceral pain [34]. It is thought that bradykinin plays an important role in migraine pain by its direct excitatory action, but clinical evidence is currently lacking.

D. Prostaglandin E_2

Prostaglandin E_2 (PGE_2) is synthesized by the cyclooxygenase (COX) pathway from arachidonic acid released from membrane phospholipids by the actions of phospholipases. PGE_2, once formed, is quickly released and act locally, due to its chemical and metabolic instability. The action of the PGE_2 is mediated via interaction with specific plasma membrane GPCR, EP receptor.

Considerable evidence implicates PGE_2 in the pathogenesis of migraine headache. Clinically, intravenous (i.v.) administration of the COX inhibitor aspirin is effective in treating acute migraine [35]. Levels of PGE_2 are elevated in the plasma, saliva, and venous blood of migraineurs during migraine attacks [36]. In addition, administration of PGs of the E series can cause migraine-like symptoms in migraineurs [37]. Electrical or inflammation-mediated stimulation of rat trigeminal ganglia *in vitro* causes delayed synthesis and release of PGE_2 from dura mater [38, 39]. This, in turn, may lead to the activation of pain-stimulating trigeminovascular afferents that innervate

and cause vasodilatation of the cranial and cerebral vasculature [40]. cAMP-coupled functional EP receptors are expressed on cultured rat trigeminal neurons, where stimulation promotes Ca^{2+}-dependent CGRP release [41]. A recent study showed that EP4 receptors mediate PGE_2-induced vasodilatation of human middle cerebral artery [42].

E. Proton

Proton production is increased in inflammation and is likely to be involved in inflammatory hyperalgesia. The mechanism of proton-induced activation of sensory neurons has been recently elucidated after the isolation of a functional cDNA encoding the capsaicin receptor (most nociceptors are characterized by their sensitivity to capsaicin, the pungent ingredient of chili peppers). Vanilloid receptor-1 (VR-1), the capsaicin receptor, is a ligand-gated nonselective cation channel that is distributed primarily in small-diameter afferent neurons. It is also located on C-fibers of human trigeminal ganglion neurons [43]. It also presents in the dura mater of the rat [44]. In addition to being sensitive to capsaicin, VR-1 responds to moderate thermal stimuli (approximately 43 °C), suggesting a heat-transduction role for this receptor [45]. VR-1 also responds to protons (H^+), suggesting that its activity might be enhanced within the acidic environment of inflamed tissues. VR-1 and related receptors might therefore play an integrative role after tissue injury based on the fact that they respond to multiple pain-producing stimuli [46]. VR-1-knockout mice show normal responses to acute noxious thermal stimuli [47]. However, hyperalgesic responses in a variety of inflammatory models are substantially attenuated or absent in these mice [48]. Because sensitization is likely to play an important role in migraine [7], VR-1 receptor may have an important role in migraine pathophysiology.

Model of trigeminovascular activation

Migraine and other forms of primary headaches have become a major interest to neuroscientists. To search for a new approach of treatment, the precise anatomical and physiological basis for migraine needs to be clarified. However it is difficult to perform the experiment in patients during a migraine attack. Therefore, an animal model of migraine has been developed. Even though there is no ideal animal model of migraine

since nobody knows whether animals do experience migraine headaches. However a model has the advantage that the complex mechanism that underlines the migraine attack can be studied in controlled condition. Several models have been used in recent years [25].

Trigeminovascular system can be activated by applying different stimuli (mechanical, electrical or chemical) in either intra- or extracranial structures innervated by the trigeminal nerve. Of particular relevance to the study of migraine has been the observation that induction of spreading depression [49], an electrophysiologic wave of depolarization hypothesized to underlie migraine aura, or stimulation of specific intracranial structures (meninges, trigeminal ganglion, superior sagittal sinus) induced Fos expression within cells in TNC. Cells in lamina I and II receive direct synaptic inputs from primary afferent fibers concerned with the transmission of nociceptive information from trigeminal receptive fields to second and higher order neurons which transmit nociceptive information to rostral centers [50].

Several techniques have been developed to study the effects of activation of the trigeminovascular system. By doing so, neuroinflammatory peptides are released antidromically from perivascular nerve fibers (CGRP, SP and neurokinin A (NKA)) [51] to promote vasodilation, activation of the endothelium with increased transendothelial transport, mast cell activation, and local platelet aggregation and adhesion [52]. All these mechanisms promote the extravasation of plasma protein into dura mater, although, not all techniques reproduce this phenomenon. At the same time, the orthodromic activation of the C-fibers releases glutamate, SP, CGRP, NKA in the TNC with activation of second order neuron [53]. Detection of Fos expression within these second order neurons is a useful tool to evaluate the activation via this polysynaptic pathway. The following is a briefly review of meningeal stimulation by topical application of an inflammatory soup (IS).

Dural inflammation model of migraine

Strassman and colleagues [54] were the first who demonstrated that chemical stimulation of dura with an IS (histamine, serotonin, bradykinin, PGE_2 at pH 5.0) sensitizes meningeal afferents, activating TNC neurons to mechanical stimuli that otherwise would have been innocuous. The excitatory and sensitizing effects of the inflammatory mediators can either be by a direct action on the primary afferent terminals or by an indirect action through release of other agents. These properties of meningeal afferents (chemosensitivity and sensitization) may well explain the intracranial mechanical hypersensitivity that characterizes some types of headaches or the headache seen in conditions such as meningitis.

In recent years, accumulating evidence has shifted the emphasis away from vascular smooth muscle and toward mechanisms related to inflammation within cephalic tissue "neurogenic inflammation theory", with concomitant activation of meningeal afferents, release of neuropeptides, vasodilatation, and extravasation of plasma proteins. In rats, these phenomena have been studied following the application of an IS containing histamine, serotonin, bradykinin, and PGE_2 to the dura. This chemical stimulation mimics the neurogenic inflammation occurring during a migraine attack. Burstein *et al.* [13] applied an IS (histamine, serotonin, bradykinin - 10^{-3} M, PGE_2 - 10^{-4} M at pH 5.0) and low-pH phosphate buffer (PB, pH 4.7) on the dorsal surface of the dura. They found that 6 of 23 trigeminal brain stem neurons were responded to IS only, 3 of 23 neurons were responded to low-pH PB only, and 7 of 23 neurons were responded to both. Levy *et al.* [55] used an IS (histamine, serotonin, bradykinin, all at 10^{-3} M, and 10^{-4} M PGE_2, pH 5.5) to induce activation and mechanical sensitization of meningeal nociceptors and central trigeminovascular neurons. They found that chemical stimulation induced long-lasting sensitization in meningeal nociceptors, manifested as an increase in ongoing firing rate, and an increase in firing produced in response to threshold mechanical stimulation of the dura at 1 hour after application. It also induced sensitization of central trigeminovascular neurons as well at 1 hour after application.

The rationale for using bradykinin, histamine, serotonin, and PGE_2 was that these agents: 1) are found endogenously, are believed to be released locally during inflammation, and are thought to be released in the vicinity of the dural sinuses by

increased plasma extravasation, and mast cell degranulation induced by neurogenic inflammation; 2) can activate and sensitize somatic and visceral nociceptive primary afferent neurons; 3) are potent algesics in humans; and 4) when applied together, they enhance each other's effects on the nociceptors. The rationale for using acidic pH is that the low pH that is found in inflamed and ischemic tissue can activate and sensitize C-polymodal and C-high-threshold mechanosensitive nociceptors and, furthermore, can have synergistic effects with some of the agents included in the IS [13].

NMDA receptor

Glutamate, the major excitatory neurotransmitter in the brain and spinal cord, exerts its postsynaptic effects via a diverse set of membrane receptors, ionotropic, and metabotropic. Ionotropic receptors directly gate ion channels and are divided into three major subclasses: AMPA (α-amino-3-hydroxyl-5-methyl-isoxazol-4-propionic acid), kainate, and NMDA (*N*-methyl-D-aspartate), named according to the types of synthetic agonists that activate them. Of these, NMDA receptors have received particular attention because NMDA receptors have critical roles in excitatory synaptic transmission, plasticity and excitotoxicity in the CNS [56, 57].

NMDA receptors display a number of unique properties that distinguish them from other ligand-gated ion channels. First, the receptor controls a cation channel that is highly permeable to monovalent ions and calcium. Second, simultaneous binding of glutamate and glycine, the coagonist, is required for efficient activation of NMDA receptor. Third, at resting membrane potential the NMDA receptor channels are blocked by extracellular magnesium and open only on simultaneous depolarization and agonist binding [56].

Native NMDA receptors are composed of NR1, NR2 (A, B, C, and D) and NR3 (A and B) subunits. Functional NMDA receptor channels require a combination of NR1, an essential channel-forming subunit, and at least one of the NR2 subunits [58]. The glutamate and glycine binding sites are located on the homologous regions of the NR2 and NR1 subunits, respectively [59, 60].

Many biophysical and pharmacological properties of the heteromeric NR1/NR2 NMDA receptor channels, such as sensitivity to magnesium block, kinetics of desensitization and offset decay, susceptibility to modulation by glycine, reducing

agents, polyamines, and phosphorylation, and affinity for agonists and antagonists, depend on the type of NR2 subunit included in a channel [57, 61].

A. NMDA receptor and pain

NMDA receptors expressed in spinal cord dorsal horn have been implicated in the activity-dependent plastic changes that lead to the generation and maintenance of central sensitization and, thereby, pathological pain [62, 63]. NMDA receptor blockade attenuates allodynia and secondary hyperalgesia, and blocks inflammatory and neuropathic pain hypersensitivity [64-66].

It has been assumed that central sensitization in the spinal cord dorsal horn is mediated by activation of postsynaptic NMDA receptors. However, one of the features unique to the spinal cord is the presence of presynaptic NMDA receptors. In fact, many small-diameter primary afferent fibers terminating in the dorsal horn express NMDA receptors, and activation of presynaptic NMDA receptors causes the release of substance P from primary afferents [67]. Rat dorsal root ganglia neurons contain NR1, NR2B, NR2C, and NR2D, but not NR2A, subunits [68]. Because SP, CGRP, and glutamate co-occur in small-diameter primary afferent terminals, presynaptic NMDA receptors can facilitate and prolong the transmission of nociceptive messages through the release of these neurotransmitters [56].

There is an evidence to suggest a role for NMDA receptors in mediating supraspinal sensitization as well. Indeed, increased NMDA receptor activation underlies inflammation-induced neuronal hyperexcitability of brainstem circuitry [69]. Transgenic mice overexpressing NR2B subunits in the forebrain exhibited enhanced responsiveness to the peripheral injection of two inflammatory agents, formalin and CFA (complete Freund's adjuvant). Neuronal Fos expression in the transgenic mice was most prominent, and significantly different from wild type controls, in the anterior cingulated and insular cortices [70].

Upon activation, NMDA receptors mediate small cation influxes (Ca^{2+} and Na^{+}) which induce the excitatory postsynaptic potential and/or modulate multiple Ca^{2+}-sensitive intracellular signaling cascades [71]. One of signaling cascades that are sensitive to NMDA receptor-mediated Ca^{2+} signals is mitogen-activated protein kinases (MAPKs). Activated extracellular signal-regulated kinase (ERK; a major subclass of MAPKs) is translocated to the nucleus and activates the transcription factors cAMP-

response-element-binding protein (CREB) and Elk-1, causing them to bind to cAMP-response elements (CRE) or serum-response elements (SRE) on gene promoter regions, respectively. This triggers transcription of immediate-early genes (IEG) such as *c-fos* and COX-2 and late-response genes (LRG) [62].

B. NMDA receptor modification

Spinal NMDA receptor activity is enhanced following peripheral inflammation, contributing to the increased activity of dorsal horn neurons [72]. Protein phosphorylation is a major mechanism for the regulation of NMDA receptor function [73]. NMDA receptors are phosphorylated by a variety of kinases, such as protein kinase A (PKA), protein kinase C (PKC), and Src type kinase.

PKC have been shown to produce a long-lasting enhancement of excitatory responses of dorsal horn neurons *in vitro* preparations. The responses of neurons in slices of the trigeminal nucleus caudalis to NMDA are enhanced after injection of PKC into the neurons [74]. PKC-mediated NMDA receptor phosphorylation potentiates channel activity [75], and neuronal responses to NMDA are enhanced by PKC, due to both an increased probability of NMDA receptor-channel opening [76] and a reduction in the tonic voltage-dependent Mg^{2+} block of the NMDA receptor [77]. Spinal PKC activation participates in the generation of pain hypersensitivity, and the potentiation of spinal NMDA receptor activity induced by peripheral inflammation is blocked by inhibition of PKC [72, 78].

There are reports that NMDA receptor protein expression changed during inflammation. Wang *et al.* [79] found that NR1 receptor was down-regulated in dorsal root ganglia after CFA injection. Change in NMDA receptor expression is also observed in pain modulatory circuitry after peripheral inflammation. In 2002, Miki *et al.* [80] demonstrated that there was an up-regulation of NR1 mRNA in rostral ventromedial medulla (RVM) started at 5 hours after CFA-induced hind paw inflammation. These findings may imply that increased excitability in the spinal dorsal horn after inflammation leads to increased NMDA receptor gene expression, increased pain modulatory neuronal activity in the RVM, and enhanced descending pain modulation.

C. NR1 receptor subunit phosphorylation

Among all NMDA receptor subunits, the NR1 subunit is an essential component for the formation of functional NMDA receptors, and this subunit is widely distributed in the central nervous system [56]. Phosphorylation of multiple sites in the cytoplasmic C terminal of the NR1 subunits is known to modulate NMDA receptor activity and to affect synaptic transmission. Phosphorylation is regulated by a number of protein kinases and phosphatases. The NR1 subunit is phosphorylated by PKA on serine-890 and -897 and by PKC on serine-896. Among three serine sites, phosphorylation of serine-897 and -896 is more common in the central nervous system. These phosphorylation events can be monitored with phosphorylation state-specific antibodies [81].

In 2003, Scott and coworkers [3] showed that endoplasmic reticulum (ER) retention mediated by the RXR (Arginine-X-Arginine) motif is an important quality control mechanism used by NMDA receptors, to ensure the proper assembly and trafficking of multimeric complexes. PKC phosphorylation at serine-896 flanking the RXR motif of the NMDA receptor NR1 subunit suppress ER retention and regulate receptor forward trafficking. This phosphorylated NR1 is rapidly dephosphorylated once ER exit is achieved. These results indicate that ER retention of NMDA receptors is tightly regulated, and suggest that phosphorylation by PKC mediates release of receptors from the ER for subsequent traffic to synapses.

In 2004, Zuo and colleagues [82] demonstrated that PKC is involved in the phosphorylation of NR1 subunits of NMDA receptors in dorsal horn neurons after intradermal injection of capsaicin (CAP). Western blots showed that pretreatment with the PKC inhibitor caused a decrease in CAP-induced phosphorylation of NR1 protein. In immunofluorescence staining, the number of phosphorylated-NR1-like immunoreactive neurons was significantly decreased on the side ipsilateral to the injection when a PKC inhibitor was administered intrathecally before CAP injection. These results strongly suggest that NR1 subunits in spinal dorsal horn neurons are phosphorylated following CAP injection, and this phosphorylation is catalyzed by PKC. Brenner and colleagues [2] also demonstrated that noxious heat applied to the hind paw of rats produces an increase in serine-896 phosphorylation of NR1 subunit in superficial dorsal horn neurons in the spinal cord. This phosphorylation was demonstrated to be mediated by activation of PKC. Phosphorylation of NR1 serine-896 is essentially

absent in the superficial dorsal horn lamina of naïve rats, but there is rapid (< 2 min) induction following a noxious but not innocuous heat stimulus. They also showed that NR1 serine-896 phosphorylation occurs in the ER, suggesting that it contributes to trafficking of the receptor from intracellular stores to the membrane. Presumably, the newly inserted NMDA receptors participate in enhancing synaptic activity and inducing central sensitization. However, it was notable that these short duration stimuli did not induce any detectable change in NR1 protein expression. These data provide evidence for an activity-dependent NMDA receptor phosphorylation at the PKC-dependent site, serine-896, in spinal cord dorsal horn.

In 2005, Caudle and colleagues [83] demonstrated that lumbar spinal cord NR1 subunits were found to be phosphorylated on serine residues within two hours of the induction of hind paw inflammation with carrageenan. The enhanced NR1 serine phosphorylation reversed within six hours. No phosphorylation on NR1 threonine or tyrosine residues was observed. Similarly, this inflammation did not alter the levels of NR1 protein expression. These data suggest that NR1 serine phosphorylation leads to an initial increase in NMDA receptor activity in the spinal cord following peripheral injury.

In 2006, Ultenius and colleagues [84], used a model of nerve injury-induced pain to assess whether alteration of NMDA receptor expression correlates with the presence of neuropathic signs. They found that the phosphorylated NR1 subunit of the NMDA receptor significantly increased in the ipsilateral dorsal horn in hypersensitive, but not in non-hypersensitive nerve-injured rats. However, they did not detect any differences in immunoreactivity in any of the non-phosphorylated NR1, NR2A, NR2B, NR2C or the NR2D subunits. These data suggest that phosphorylation of the NMDA receptor 1 subunit is correlated to the presence of signs of neuropathy (stimulus evoked pain-like behavior) and possibly also to persistent pain following nerve injury. This may represent a mechanism involved in spinal sensitization.

Fos

The *c-fos* is an early-response proto-oncogene. It is rapidly and transiently expressed in response to noxious inputs in the central nervous system. The immunocytochemical identification of Fos protein has been used as an indicator of

activation of the nociceptive neurons [85]. In migraine pain models, Fos in TNC is activated by either electrical stimulation of the trigeminal ganglia, chemical stimulation of the meninges, electrical or mechanical stimulation of the superior sagittal sinus, or by induction of cortical spreading depression. There is evidence that several receptors, including NMDA receptor, modulate Fos expression within TNC [86]. In the Fos model, the selective NMDA antagonist significantly and dose-dependently reduces Fos expression within TNC induced either by capsaicin or formalin [87, 88].

Double immunocytochemical study shows that about 25% and 55% of the Fos-immunoreactive neurons that are found in lamina I–II and lamina V show NMDA receptor immunoreactivity, while about 4% and 11% of NMDA-receptor-immunoreactive neurons in these two regions show Fos immunoreactivity, respectively [89]. NMDA receptor regulates Fos expression via ERK-CREB cascade as described earlier.

Serotonin and pain modulatory system

Central serotonergic pathways have been implicated in mechanisms of pain modulation. The entire serotonergic innervation of the spinal cord is derived from supraspinal sources. Cell bodies of serotonergic neurons are restricted to discrete clusters of cells located along the midline of the brainstem. However, their axons innervate nearly every area of the central nervous system. Dahlstrom and Fuxe [90] described nine groups of serotonin-containing cell bodies, designated B1 to B9, which correspond for the most part of raphe nuclei. In this regard, a modest proportion of serotonergic neurons from the dorsal raphe nucleus send collaterals to the spinal cord and the trigeminal nucleus [91, 92]. However, the predominant source of serotonergic input to the spinal cord and trigeminal nucleus arises within the vicinity of the RVM and, most prominently, from the nucleus raphe magnus (NRM) [93, 94]. This spinal termination plays a significant role in pain modulation, shaping the firing pattern of thalamic projection neurons as well as the primary afferents [6].

Numerous axo-somatic and dendritic contacts of serotonergic terminals are seen in the dorsal horn, some of which reveal contact between serotonergic terminals and dorsal horn neurons projecting to the thalamus [95, 96]. In view of anatomical evidence for 5-HT receptors on primary afferents, there is remarkably little evidence for

apposition of serotonergic fibers at central primary afferent terminals in the dorsal horn and trigeminal nucleus [97, 98]. Nevertheless, primary afferent activity may be modulated via local inhibitory interneurons which receive an input from descending serotonergic pathways [95, 99].

The actions of 5-HT within the spinal cord are complex. There is evidence for both pro-nociceptive and anti-nociceptive effects of 5-HT in behavioral and electrophysiological paradigms, and these are variously mediated by different 5-HT receptor subtypes [100]. Activation of spinal 5-HT_{1A} receptors produces pro-nociceptive effects while 5-HT_{1B} and 5-HT_3 receptors produce anti-nociception. Activation of 5-HT_2 receptors produces anti-nociception which can be preceded by pro-nociceptive responses [101, 102].

Serotonin depletion and migraine

It has long been known that 5-HT plays an important role in migraine pathogenesis [103]. In 1969, Anthony showed that the administration of reserpine, which cause a fall in plasma 5-HT, may trigger typical migraine attacks in migraineurs [104]. Ferrari *et al.* [105] demonstrated that attacks of migraine coincided with a fall in platelet 5-HT as well as increase in plasma 5-HT. Lowered level of platelet 5-HT is thought to reflect monoamine depletion in brainstem nuclei. Sicuteri [106] demonstrated that p-chlorophenylalanine (PCPA), a 5-HT depletor, provoked systemic pain after 20 to 40 days in 4 of 18 migraineurs. The administration of 5-HT or 5-HT agonists (mainly $5\text{-HT}_{1B/1D}$ agonists) can abort the attack of migraine. It may hypothesized that the descending inhibitory serotonergic pathway involved in the control of pain is defective in migraine sufferer, and that central 5-HT depletion accentuates the perception of pain by reducing the efficacy of the endogenous pain control system and induces migraine attacks [4, 5]. Panconesi and Sicuteri [107] proposed that 5-HT neuronal depletion cause central 5-HT hypersensitivity in migraine patients. Fozard [108] also proposed that in migraine patients, brainstem 5-HT neurons are partially depleted, causing postsynaptic (endothelial) $5\text{-HT}_{2C}/5\text{-HT}_{2B}$ hypersensitivity. These may predispose migraineurs to migraine attacks.

In 2000, Srikiatkhachorn *et al.* [103] found the role of hyposerotonin in the modulation of a cranial vascular response to nitric oxide (NO). They demonstrated that

5-HT depleted animals enhanced responses of meningeal and cerebral microvessels to NO. They also found that low 5-HT animals produced more considerably changes in cerebral microvessel, characterized by focal ballooning of endothelial cells, increased microvillous formation, and increased endothelial endocytosis, than control animals. This may be an explanation for the supersensitivity to NO observed in patients with migraine. These findings imply a relationship between 5-HT depletion and the development of migraine attack.

In 2005, Drummond [109] found that control subjects that consumed an amino acid drink that omitted L-tryptophan (thereby reducing brain 5-HT synthesis) boosted dizziness, nausea, and the illusion of movement to levels that approached those of migraineurs. Thus, reduced brain 5-HT activity may promote vestibuloocular disturbances during motion sickness and attacks of migraine. In 2006, Drummond [110] further investigated the sensitivity to light in migraine sufferers and control subjects after consumption of an amino acid drink which contained L-tryptophan (balanced amino acid condition) or of a drink that omitting L-tryptophan which produced a short-term reduction in brain 5-HT synthesis (tryptophan depletion condition). Migraine sufferers reported more intense nausea, headache, glare- and light-induced pain than controls. In addition, glare- and light-induced pain was greater in the tryptophan depletion condition than in the balanced amino acid condition, in both migraine sufferers and controls. Eight hours after the amino acid drink, tryptophan depletion augmented headache in migraine sufferers and aggravated nausea in migraine sufferers and controls. These findings suggest that a reduction in brain synthesis of 5-HT intensifies photophobia and other migrainous symptoms and thus might contribute to the pathogenesis of migraine.

In 2006, Supornsilpchai *et al.* [111] found that the development of cortical spreading depression (CSD) waves by application of KCl was enhanced in low 5-HT state. The area under curve of each CSD wave and the number of CSD waves occurring within 1 hour were greater in low 5-HT group. No significant change in peak amplitude and duration of CSD wave was observed. The numbers of Fos-immunoreactive cells on ipsilateral and contralateral TNC were significantly greater in the low 5-HT group than those of the controls. These findings indicate that 5-HT depletion enhances CSD-induced trigeminal nociception by increasing the cortical excitability and sensitivity of trigeminal nociceptive system.

Serotonin depletion and chronic daily headache (CDH)

CDH features increased headache frequency, expansion of headache area, and cutaneous allodynia. Experimental and clinical findings suggest a primary role of central sensitization in the pathogenesis of CDH. Suppression of the endogenous pain control system can facilitate the process of central sensitization [6]. Evidence of such suppression in patients with chronic daily headache includes decreased platelet 5-HT, up-regulation of 5-HT_{2A} receptors. Alterations in the 5-HT system have been demonstrated in patient with medication-induced CDH [112, 113]. Compared with patients with migraine, patients with CDH have a lower level of platelet 5-HT and greater density of 5-HT_{2A} receptors. These changes can be reversed after drug withdrawal, and normalization of platelet 5-HT and its receptor correlates with clinical improvement.

Chronic analgesic exposure can alter endogenous 5-HT-dependent system of pain modulation by inducing a low 5-HT state. The low level of 5-HT subsequently leads to up-regulation of the pro-nociceptive 5-HT_{2A} receptor. Stimulation of these highly expressed receptors and reduction of pain modulation may enhance the process of central sensitization. Activation of 5-HT_{2A} receptor may potentiate nociception by enhancing the release of SP from the primary afferent [114]. Transduction cascade of the 5-HT_{2A} receptors is hydrolysis of phosphoinositol. Thus, 5-HT_{2A} receptor activation causes elevation of intracellular calcium and subsequent activation of various kinase enzymes, such as calcium-calmodulin-dependent protein kinase II, PKA, and PKC. Phosphorylation of neurotransmitter receptors, ion channels, and transcription proteins by these kinases is an important step in the development of central sensitization [6].

In 2002, Srikiatkhachorn *et al.* [29] demonstrated that 5-HT_{2A} agonist, DOI, administration led to shortening of tail flick latancy and to an increase in the number of Fos-immunoreactive neurons in TNC. DOI also produced long-lasting cerebral hyperemia associated with the enlargement of perivascular neural nitric oxide synthase (nNOS)-immunoreactive nerve fibers and increased nNOS-immunoreactive neurons in trigeminal ganglia and TNC. These results suggest that activation of the 5-HT_{2A} receptor leads to an enhancement of NO production in trigeminovascular pathway. Up-regulation of this pronociceptive receptor can increase headache attacks and contribute to the development of CDH.