Chapter 18

Prostanoids and Other Inflammatory Mediators

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Introduced by Bayer in 1899, acetylsalicylic acid (aspirin [ASA]) is today the most extensively prescribed analgesic, antipyretic, and anti-inflammatory agent, with 120 billion tablets consumed annually (68). The efficacy of ASA (and other nonsteroidal anti-inflammatory drugs [NSAIDs]) for headache relief has been extensively assessed in placebo-controlled studies (33,40,49,62,73). Sir John Vane was awarded the Nobel Prize for Physiology or Medicine in 1982 for his discovery that aspirin's major therapeutic and adverse effects could be explained by inhibition of prostaglandin synthesis by inactivation of the key enzyme, cyclooxygenase (COX) (67). However, despite being among the most commonly used antimigraine drugs, the precise mechanism of action of these agents in migraine headache therapy is still not clear.

We now know that at least three COX isoforms exist (Figure 18-1). COX-1 is the constitutive enzyme producing prostaglandin and thromboxanes involved in physiologic activities like cytoprotection and platelet aggregation, whereas COX-2 appears to be preferentially expressed in inflamed tissues. Recently, a COX-1 splice variant, named COX-3, has been cloned and found to be abundantly expressed in mature brain and spinal cord (10). COX-3 inhibition in the brain may be responsible for analgesic and antipyretic effects of certain NSAIDs. Whereas ASA inhibits all three COX isoforms, a new class of inhibitors termed *coxibs* selectively inhibit COX-2. Acetaminophen blocks mostly COX-3. ASA is unique because it inactivates COX by irreversible acetylation of serine residue in the active site of the enzyme; other NSAIDs are competitive reversible inhibitors of COX. Prostaglandins sensitize free nerve endings (pain receptors) to numerous inflammatory mediators and, when injected directly into the brain, induce fever and pain. ASA, by inhibiting all isoforms of COX, can be considered both a peripherally and centrally acting analgesic. The central mechanism of action is supported by autoradiographic studies that showed high-affinity binding of ASA to nociceptive structures in the brain (26). Additionally, in animal studies, intravenous ASA inhibits central trigeminal neurons in the dorsal horn of the upper cervical spinal cord after stimulation of the sagittal superior sinus (35).

Because of the established efficacy of NSAIDs in migraine therapy, prostanoids and their potential targets are highly relevant to migraine pathophysiology. However, various other mediators are important for inflammatory processes, and might provide additional therapeutic opportunities in migraine. They are therefore discussed in the following section.

INFLAMMATORY MEDIATORS

Commonly, inflammation occurs as a defensive response to invasion of the host by foreign, particularly microbial, material. Responses to mechanical trauma, toxins, and neoplasia may also result in inflammatory reactions. Microscopically, inflammation involves a complex series of events, including dilation of arterioles, capillaries, and venules, with increased permeability and blood flow, as well as exudation of fluids, including plasma proteins. The accumulation and subsequent activation of leukocytes are central events in the pathogenesis of most forms of inflammation. The development of inflammatory reactions is controlled by various mediators, including free radicals, the complement system, kinins, cytokines, and prostanoids.

Free radicals are atoms or molecules that readily react with other cellular structures because they contain unpaired electrons. Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, and hydroxyl species, are normal byproducts of cellular electron transfer reaction, ordinary metabolic processes, and immune system responses (the secretion of reactive oxygen and

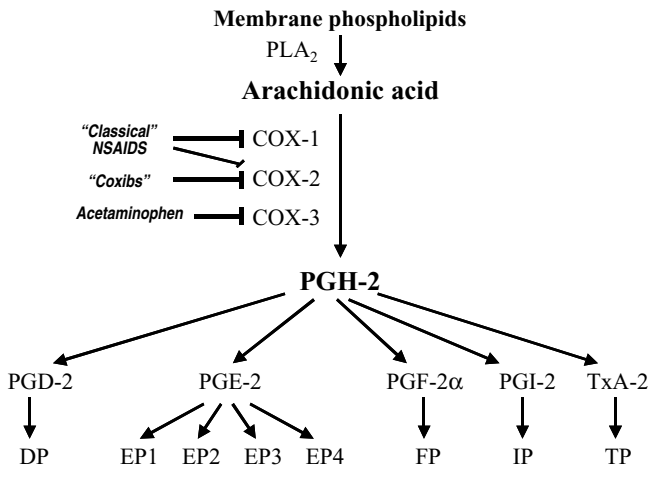

FIGURE 18-1. Summary of biosynthetic routes to the major prostaglandins and thromboxane A2. Arachidonic acid is generated from membrane phospholipids via the action of two types of phospholipase A2 (a secreted 14 kilodalton form, and the type IV cytosolic 85 kilodalton isoform [6]). Arachidonic acid is the converted to the prostaglandin endoperoxides PGG_2 (not shown) and PGH_2 by two enzymes, COX-1 and COX-2. A COX-1 splice variant, named COX-3, has been cloned and found to be abundantly expressed in mature brain and spinal cord (10). PGH_2 spontaneously decomposes in aqueous solution to form a mixture of PGD_2, PGE_2, and PGF_2. However, distinct enzymes catalyze the formation of each of these prostaglandins, as well as TXA_2 and PGI_2. The cellular expression pattern of each of these enzymes may influence the type of prostaglandin produced by a particular cell. Prostaglandins are liberated from cells and bind to a family of G protein coupled receptors (see Table 18-2).

nitrogen free radical species by inflammatory cells is a major mechanism for attacking foreign substances). Free-radical–generating substances can also be found in food or drugs. ROS are important in inflammation and may play a role in nociceptor activation. For instance, hydrogen peroxide has been shown to enhance the effects of other inflammatory mediators (bradykinin, prostaglandins), and nitric oxide ([NO] a reactive nitrogen species) induces a delayed burning pain upon intradermal injection (29). NO donors can activate sensory fibers directly, causing the release of CGRP (72). There is also strong evidence that NO is involved in the migraine pathogenesis (see below).

The *complement* comprises several families of proteins activated in sequence when cells are exposed to a foreign substance. Once the proteins are activated, nine of these proteins come together to form the membrane attack complex (MAC). When assembled on a cell membrane, MAC forms a ring-like structure that allows the movement of ions and small molecules into and out of the cell, resulting in cell damage. In addition to these toxic effects, complement anaphylatoxins C3a and C5a initiate local inflammatory responses and various complement proteins also activate phagocytic and endothelial cells. Interestingly, plasma completely loses its ability to excite trigeminal neurons after heat inactivation, suggesting that the complement system may be involved in the excitatory nociceptive transmission in the trigeminal system (21).

Kinins (bradykinin, kallidin) exert a number of pro-inflammatory effects via two distinct receptors (B1 and B2), including the release of prostanoids, cytokines, and free radicals from a variety of cells. 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 mechanical stimuli (69).

Cytokines are signaling proteins that are secreted by various types of immune cells. The central role of cytokines is to control the direction, amplitude, and duration of the inflammatory response. Pro-inflammatory cytokines (interleukin [IL]-1, IL-6, tumor necrosis factor [TNF]-α, transforming growth factor-β) are produced predominantly by activated immune cells and are involved in the amplification of inflammatory reactions. Anti-inflammatory cytokines (IL-4, IL-10, and IL -13) are involved in the reduction of inflammatory reactions. IL-1β, IL-6, IL-8, and TNF-α can indirectly induce powerful hyperalgesia by causing prostanoid release, increasing the expression of nerve growth factor (NGF) and bradykinin receptors, or affecting sympathetic fibers (15,16,34).

Prostanoids are a group of lipid mediators that consist of the prostaglandins and thromboxanes. In response to cell stimulation, prostanoids are synthesized by the COX pathway from arachidonic acid released from membrane phospholipids by the actions of phospholipases. Prostanoids, once formed, are quickly released to the outside of cells. Because of their chemical and metabolic instability, prostanoids are believed to act in the vicinity of their sites of production. Thus, they are "short-range hormones," maintaining local homeostasis in a variety of tissues and cells. Although some prostaglandins have anti-inflammatory effects (they decrease inflammation, increase oxygen flow, prevent cell aggregation, and decrease pain), others are known to have pro-inflammatory effects.

MIGRAINE AS AN INFLAMMATORY DISORDER

Despite considerable research into the pathogenesis of idiopathic headaches such as migraine, their underlying pathophysiologic mechanisms remain poorly understood. A recent meta-analysis of clinical literature published between 1966 and 1999 found only about 45 clinical investigations reporting alterations of immune function in migraine patients (37). Changes of serum levels of complement and immunoglobulins, histamine, cytokines, and

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immune cells were reported in some of these studies, but in most cases were not corroborated by others. The inflammatory processes possibly involved in migraine are therefore unlikely to be of immune origin.

According to modern theories of migraine pathogenesis, initial activation of intracranial perivascular sensory fibers supplying the dura mater might result from exposure to endogenous (brain-generated or bloodborne) algogenic chemicals. Activation of meningeal nociceptors causes these sensory fibers to release neuropeptides, including substance P and calcitonin gene-related peptide (CGRP) (43). This initiates neurogenic inflammation in the dura mater, leading to a further secretion of inflammatory agents such as serotonin, histamine, bradykinin, and prostaglandins. After application of a cocktail of these inflammatory mediators to the dural sinuses, rat meningeal primary afferent neurons rapidly become mechanically hypersensitive (i.e., neurons that showed no or only minimal response to a small dural indentation before the chemical irritation show a strong response minutes after application of these inflammatory mediators) (63). Similarly, this cocktail can cause central trigeminal neurons receiving convergent input from the dura and the skin to lower their thresholds to mechanical stimulation of the dura and to mechanical and thermal stimulation of the skin. Application of lidocaine to the dura abolishes the response to dural stimulation, but has minimal effect on the increased response to cutaneous stimulation, suggesting involvement of a central mechanism in maintaining the sensitized state, which can last up to 10 hours (8). On the basis of these studies, Burstein (7) has proposed that (a) sensitization of both peripheral and central trigeminovascular neurons accounts for the intracranial hypersensitivity observed in migraineurs, and (b) sensitization of central but not peripheral trigeminal neurons is responsible for the extracranial hypersensitivity (extracranial tenderness and cutaneous allodynia) often seen in these patients.

Nitric oxide, a short-lived vasodilator and reactive nitrogen species, has been implicated in the genesis of migraine. Indeed, headache is a well-known side effect of NO donors such as nitroglycerin or sodium nitroprusside. Intravenous infusion of nitroglycerin causes migraineurs, and not control subjects, to develop a delayed migraine attack (47). Furthermore, the NO synthase inhibitor N^G-methylarginine (L-NMMA) effectively improves spontaneous migraine headaches and associated symptoms such as phono- and photophobia (39). These human studies implicate NO in the genesis of headache. In addition, there is also abundant evidence about NO in pain generation and maintenance derived from animal models (41).

Based on these data indicating that NO may participate in the early and late phases of a migraine attack, several groups have studied the effects of NO (administered as the NO donor nitroglycerin) on cephalic structures (38,53,64). Recently, nitroglycerin infusion has been shown to cause a delayed expression of inducible nitric oxide synthase (iNOS) in rat meningeal macrophages (56,57). This iNOS induction is preceded by the appearance of IL-1β in the dura mater, and is followed by mast cell degranulation, IL-6 expression, and plasma protein extravasation (blocked by administration of an iNOS inhibitor), indicating the occurrence of nitroglycerin-induced inflammatory events in the meninges. It is likely that a similar inflammatory response occurs in more spontaneous types of migraine attacks.

RECEPTORS FOR INFLAMMATORY MEDIATORS

G protein-coupled receptors (GPCRs) are the most important target for the pharmaceutical industry, as is indicated by the fact that they are the site of action of 52% of all medicines available today (48). This is probably because of the relative ease of designing low-molecular-weight agents blocking the interaction of small transmitter molecules and their receptors. GPCRs for inflammatory mediators (prostaglandin and kinins, but also chemokines) are important mediators of peripheral sensitization. Because of their potential importance to migraine drug therapy, their pharmacology is reviewed in this section.

Kinin Receptors

Kinins are 9-11 amino acid peptides acting on blood vessels and involved in cardiovascular regulation, inflammation, and pain. Their effects are mediated by two GPCR subtypes: B2 (constitutively expressed) and B1 receptors (inducible and upregulated in the presence of cytokines, endotoxins or tissue injury; Table 18-1). These receptors have been defined based on pharmacologic criteria: bradykinin, kallidin, and T-kinin are endogenous agonists for B2 receptors, and Des-Arg^9-bradykinin and Des-Arg^{10}-kallidin prefer B1 receptors (54,55). B1 receptors exert both protective (e.g., in multiple sclerosis and septic shock) and negative effects (pain, edema, and inflammation) and play a major role in the chronic phase of the pain and inflammatory responses. In contrast, B2 receptors are important mediators of the acute phase of inflammation (arterial relaxation, venoconstriction, increased permeability) and of somatic and visceral pain. Autoradiographic studies show that B2 receptor binding sites predominate in the superficial laminae of the dorsal horn, particularly on the terminals of Aδ and C fibers (14), and intrathecal bradykinin administration results in both antinociception (via activation of B2 receptors on the terminals of sensory fibers) and nociception (through the release of noradrenaline from inhibitory neurons projecting to the dorsal horn) (14). It is therefore

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TABLE 18-1 Properties of Kinin Receptor Subtypes and Primary Structure of Mammalian Kinins

Subtype	*Preferred Agonists*	*Signaling*	*Expression*
B1	Des-Arg^{9}-bradykinin Des-Arg^{10}-kallidin	cAMP increase PLC, PLA_2	Inducible
B2	Bradykinin Kallidin T-kinin	PLA_2, PLC, MAPK	Constitutive

Bradykinin (BK): Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-**Arg**-OH
Kallidin (KD): Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-**Arg**-OH
T-kinin (TK): Ile-Ser-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-**Arg**-OH
Des-Arg^{10}-KD: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OH
Des-Arg^{9}-BK: Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OH
Des-Arg^{11}-TK: Ile-Ser-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OH

likely that the stimulation of central B2 receptors plays an important role in migraine pain.

Prostaglandin Receptors

Prostaglandins are important mediators of pain and inflammation, and considerable evidence implicates their involvement in the pathogenesis of migraine headache. Clinically, intravenous administration of the COX inhibitor aspirin is effective in treating acute migraine (19). Furthermore, NSAIDs such as paracetamol, ibuprofen, ketoprofen, and diclofenac, which can inhibit prostaglandin synthesis, have been shown to be 2- to 3-fold more effective than placebo in treating migraine and tension headache (12,18,36,52). Levels of prostaglandin (PG) E_2 are elevated in the plasma, saliva, or venous blood of migraineurs during migraine attacks (45,60,66). In addition, migraine-like symptoms can be induced in migraineurs by the exogenous administration of prostaglandins of the E series (9,51). Finally, a major side effect of the IP receptor agonist iloprost is headache (25,28). Taken together, these observations suggest that not only inhibiting prostanoid synthesis, but also blocking prostanoid receptors, would be an effective approach to treat migraine headache.

The actions of the five naturally occurring prostanoid metabolites of arachidonic acid (PGD_2, PGE_2, PGF_2, PGI_2, and thromboxane A_2) are mediated via interaction with specific plasma membrane GPCRs. Five major subdivisions of the prostanoid receptor family, termed DP, EP, FP, IP and TP, have been defined on the basis of their pharmacologic sensitivity and molecular identity (13,65) (Table 18-2). In smooth muscle, FP and TP receptors are functionally associated with contractile responses; DP and IP receptors mediate relaxation. EP receptors have been pharmacologically classified further into EP_1, EP_2, EP_3, and EP_4 subtypes, based on their relative sensitivities to a range of naturally occurring and synthetic agonists and antagonists. Whereas EP_1 and EP_3 receptors are coupled to Ca^{2+} mobilization and the inhibition of cyclic adenosine monophosphate (cAMP) via Gq/Gi-proteins and

TABLE 18-2 Properties of Prostanoid Receptor Subtypes

Subtype	*Potency Order*	*G Protein*	*Signaling*	*Alternative Splicing*
DP	D>>E>F>I,T	Gs	cAMP increase	None
EP				
EP_1	E>F,I>D,T	Gq/11	Ca^{2+} increase	2 (rat)
EP_2	E>F,I>D,T	Gs	cAMP increase	None
EP_3	E>F,I>D,T	Gq/11, Gi/o, Gs	cAMP increase Ca^{2+} increase cAMP decrease	3 (mouse) 4 (rat) 8 (human)
EP_4	E>F,I>D,T	Gs	cAMP increase	None
FP	F>D>E>I,T	Gq/11	Ca^{2+} increase	2 (cow)
IP	I>>D,E,F>T	Gs, Gq/11	cAMP increase Ca^{2+} increase	None
TP	T,H>>D,E,F,I	Gq/11, Gi/o, Gs	cAMP increase Ca^{2+} increase cAMP decrease	2 (human)

D: PGD_2, **E:** PGE_2, **F:** $PGF_{2\alpha}$, **H:** PGH_2, **I:** Prostacyclin or PGI_2, **T:** Thromboxane

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mediate smooth muscle contraction, EP_2 and EP_4 receptors are coupled to the stimulation of adenylyl cyclase via Gs proteins, and have previously been shown to exert relaxant effects on vascular smooth muscle (46).

Clinically effective antimigraine drugs (e.g., 5-HT_1 receptor agonists) might act via inhibition of trigeminovascular afferents and neuropeptide release (43) or vasoconstriction of the cerebral and meningeal vasculature (22). Electrical- or inflammatory-mediated stimulation of rat trigeminal ganglia in vitro has been demonstrated to cause a significant release of the neuropeptide CGRP from sensory nerve fibers, and a delayed synthesis and release of PGE_2 from dura mater (20,58). This, in turn, can lead to the activation of pain-stimulating trigeminovascular afferents that innervate and cause vasodilatation of the cranial and cerebral vasculature (75). cAMP-coupled, functional EP prostanoid receptors have recently been demonstrated to be present on cultured rat trigeminal neurons, where stimulation mediates Ca^{2+}-dependent CGRP release (32). A recent study showed that EP_4 receptors mediate PGE_2-induced vasodilatation of human middle cerebral artery, in addition to IP receptors mediating relaxation and TP receptors mediating contraction (17). In this context, it is worth mentioning that considerable species differences have been reported regarding the effects of prostanoids on isolated cerebral blood vessels. PGE_2 has been demonstrated to weakly relax 5-HT precontracted feline basilar and middle cerebral arteries (74), but to contract canine, rabbit, and human basilar arteries (44,50). In conclusion, endogenously produced prostaglandins, most notably PGE_2, seem to play a direct role in the pathophysiology of migraine by stimulating CGRP release from trigeminal afferents. Selective EP_4 antagonists may therefore be therapeutically beneficial.

Cytokines Receptors

Cytokine refers to a multiplicity of soluble extracellular proteins or glycoproteins that mediate or modulate pleiotropic pro- and anti-inflammatory responses via specific receptors on target cells (1). Cytokines have been assigned to various family groups based on the structural homologies of their receptors. With few exceptions, these receptors are heterodimers with one receptor subunit binding cytokines and the other transducing signals into the cell. Type I cytokine receptors share a conserved amino acid sequence (WSXWS) with four paired cysteines in their extracellular domain. Type II cytokine receptors, which bind interferons, share sequence homology with type I receptors, but have additional cysteine pairs and conserved proline and tyrosine residues. The third type of cytokine receptors binds IL-1α and IL-1β and contains immunoglobulin domains. The TNF receptor family (type IV) is characterized by type I membrane proteins with a cysteine-rich region in the extracellular domain. The fifth type of cytokine receptors binds CC or CXC chemokines, and is among the large family of seven transmembrane domain GPCRs.

More than one cytokine usually elicits a particular biologic activity (redundancy). For instance, IL-1 and TNF-α exert overlapping effects on adhesion molecules, accumulation of leukocytes at the sites of inflammation, acute protein synthesis, and angiogenesis. Several mechanisms of cytokine–receptor interactions have been described. Cytokines usually have a short action radius, and the most common mechanism of action involve autocrine and paracrine interactions. Whereas most cytokines function in a paracrine manner, autocrine pathways are important because they enable amplification of inflammatory responses. Cytokines released into the peripheral circulation may also function in an endocrine manner (e.g., IL-1, IL-6, and TNF-α can induce hepatic cell acute phase protein production and generalized septic shock syndrome).

Cytokines appear to have a role in the sensory hypersensitivity associated with inflammation (15,23,24,59,70,71). This action is commonly indirect, by stimulating the release of agents that act on neurons (59). Recently, it has also been shown that cytokines and their receptors are widely expressed in the central nervous system by all types of cells, including neurons, indicating that they might act on neuronal receptors (11). The levels of neurotrophin NGF are elevated during inflammation, induced to a large extent by IL-1β (59), and there is considerable evidence for a major role of NGF in mediating inflammatory hyperalgesia. This effect is partly caused by NGF's indirect action on sympathetic neurons (2,76) and by stimulation of mast cell degranulation (30,76), but is mainly a result of its interaction with the high-affinity NGF receptor trkA (76), which is expressed on a subpopulation of sensory neurons (4). Similarly, the elevation of TNF-α in inflammation, by virtue of its capacity to induce IL-1β and NGF, may contribute to the initiation of inflammatory hyperalgesia (77).

As mentioned, a cytokine-mediated inflammatory response might account for the delayed migraine headache following nitroglycerin infusion (56,57). It is possible that other exogenous factors, found in air, water, food, or drugs, activate similar pathways. Several lines of evidence suggest that migraine could also be caused by endogenous factors. Migraine patients have been postulated to have an increased cortical hyperexcitabilility, based on the results of transcranial magnetic stimulation experiments (3,5). There is also increasing evidence to indicate that migraine aura arises from cortical spreading depression (CSD) (27), migraine headache being caused by the ensuing trigeminal-induced meningeal inflammation. Interestingly, increased TNF-α and IL-1α mRNA levels are found in ipsilateral rat cortex within 4 hours of KCl-induced CSD (31). These results further suggest that pro-inflammatory cytokines may be involved in the pathophysiology of migraine headache.

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CONCLUSION

The possibility that migraine headache might be the result of sterile inflammatory events in the meninges provides a basis for the therapeutic action of NSAIDS in migraine (56). The discovery of the COX-2 pathway has led to the development of a new generation of selective inhibitors with an improved side effect profile. These agents seem to be effective and generally well-tolerated acute therapies in patients experiencing migraine with or without aura (61). However, despite these recent findings, very few antimigraine therapies, beside COX inhibitors, are based on inhibition of inflammatory pathways. This chapter has highlighted several potential targets (enzymes or receptors) that could provide the basis for the development of novel antimigraine agents (e.g., various prostaglandin receptor subtypes, kinin, and cytokine receptors). Novel antiinflammatory drugs targeting these sites are currently being developed for indications other than migraine (42). It is quite possible that some of them will be effective antimigraine drugs.

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