Modulation of the nitroglycerin-induced activation of second order trigeminal neurons in the rat

Summary of Ph.D. Thesis

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Original publications related to the Ph.D. thesis

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III. **Varga H**, Párdutz Á, Vámos E, Bohár Z, Bagó F, Tajti J, Bari F, Vécsei L (2009) Selective inhibition of cyclooxygenase-2 attenuates nitroglycerin-induced calmodulin-dependent protein kinase II alpha in rat trigeminal nucleus caudalis, *Neurosci Lett.* **451**, 170-3.

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List of abbreviations

5-HT	serotonin
CamKII	calmodulin dependent protein kinase II
cGMP	cyclic guanosine monophosphate
CGRP	calcitonine gene-related peptide
COX	cyclooxygenase
COX-1	cyclooxygenase-1
COX-2	cyclooxygenase-2
COX-3	cyclooxygenase-3
Ir	immunoreactive
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
NSAID	non-steroid anti-inflammatory drug
NTG	nitroglycerin
s.c.	subcutaneous
TNC	spinal portion of the caudal trigeminal nucleus

I. Introduction

Migraine headache is the most common neurological disorder, affecting up to 12% of the population. Among others gonadal hormones are important modulators of migraine. After puberty women are three to four times more frequently affected by this headache disorder.

Migraine is commonly known as a throbbing unilateral head pain that is readily aggravated by routine physical activities. The clinical definition of migraine includes a host of transient neurological symptoms other than pain, namely, nausea, photophobia, phonophobia, osmophobia, fatigue, and numerous disturbances in autonomic, mental, sensory and motor functions. Numerous factors are putatively involved in the etiology of migraine such as susceptibility to particular stimuli (stress, nitrated foods etc) or changes within the central nervous system. The neural mechanisms underlying the development of migraine attacks are not yet fully understood.

The throbbing pain experienced in migraine was first attributed to the pulsations of abnormally dilated vessels. Nociceptive inputs generated from the pain-sensitive extracerebral vessels are then sent via the trigeminal ganglion and subsequently the spinal trigeminal nucleus caudalis (TNC) to higher centers involved in pain processing. Nevertheless vasodilatation per se could not account for a number of observations in migraine sufferers and a neuronal origin of the pain was suggested. According to this hypothesis activation of meningeal nociceptors could cause local inflammation, which in turn, further stimulates perivascular nociceptive trigeminal fibers. Nitric oxide (NO) may have a crucial role in this process as an important mediator in the initiation or the propagation of a neurogenic cranial vessel inflammatory response that might eventually result in a migraine attack, and in the changes in cerebral blood flow during migraine.

Another important asset of migraine pathology is the presence of sensitization process during the attack. Strassman et al. showed that meningeal primary afferent neurons can become mechanically hypersensitive upon exposure of their dural receptive field to inflammatory agents. In humans, such mechanical hypersensitivity could mediate the throbbing pain of migraine and its worsening during coughing, bending over, or other physical activities that increase intracranial pressure.

The sensitization process is not only restricted to the peripheral nerves but involves the central nervous system. The central sensitization hypothesis, which proposes that altered processing of sensory input in the brainstem, principally the trigeminal nucleus caudalis, could account for many of the temporal and symptomatic features of migraine. Central sensitization involves the TNC, as it is associated with abnormal neuronal excitability in this nucleus. Several studies suggest that the NO donor nitroglycerin (NTG) may have a hyperalgesic effect and that sensitization of pain pathways in the spinal cord may be caused by – or associated with – the generation of NO. High doses of NTG reduced tail flick latency in rats and it seems that prolonged elevation of NO in the spinal cord is necessary to maintain central sensitization after it has been established. In several models of neuropathic and inflammatory pain, neuronal nitric oxid synthase (nNOS) inhibition reduces central sensitization and, indeed, pain responses in these models are increased by NO donors. The strongest evidence for the key-role of NO in the etiology of migraine stems from observations in migraine sufferers and led to the NO hypothesis of migraine. Systemic administration of NTG, a NO donor, induces an immediate headache in humans. In migraineurs, this headache is more severe, lasts longer, and can continue to or be followed by a specific attack of migraine without aura. A biphasic headache can also be triggered by NTG in patients suffering from chronic tension-type headache. This effect is very likely to be related to NTG-derived NO. The role of NO is also supported by the fact that other drugs that are able to induce migraine such histamine, reserpine or the serotoninergic antagonist mCPP all have in common to be associated with the release of endogenous NO. The infusion of NTG in human induces an immediate throbbing headache in healthy subjects, while most migraine patients experience a more severe delayed headache, that is identical to spontaneous migraine attacks except the aura. Due to the vasodilating effect of NO, the immediate headache is a consequence of the selective action of NTG on extra- and intracranial blood vessels, principally the dural arteries and large penetrating cerebral arteries, while the delayed headache might be mainly due to an effect of NO on neuronal function. Although the half-lives of NTG and NO are very short in vivo, the migraine attack occurs several hours after NTG infusion. Thus it appears that NO is a cause of migraine through mechanisms that develop over a long period of time. This is consistent with the possibility of a delayed and sustained production of NO by nitric oxide synthases (NOSs) in a large number of tissues. The importance of endogenous NO production during the

headache phase of migraine has been evidenced in the study of Lassen et al. where the inhibition of NOS relieved the symptoms of spontaneous migraine with high efficacy.

Results from animal experiments supports that NTG activates the pain-mediating TNC neurons, as the administration of NTG in the rat significantly enhanced the number of Fos-immunoreactive neurons in brain areas involved in sensory nociceptive perception, including the TNC. This effect was maximal after a delay of 4 hours, congruent with the delay of NTG induction of migraine in human. Furthermore in the animal model a large number of the activated neurons also exhibited NOS immunoreactivity. In neurons of the lower TNC (including the upper cervical spinal cord), where most of the trigeminal nociceptors project, NTG also increases the expression of nNOS. The most likely explanation for this increased nNOS immunoreactivity is the secondary activation of second order nociceptive neurons and/or interneurons because of excitation of their peripheral afferents. The increased nNOS level in the second order trigeminal neurons can be associated with a central sensitization phenomenon, which is characteristic in migraineurs. This effect is inhibited by pretreatment with acetyl-salicylate, but not with sumatriptan, indicating that prostanoids are involved in this process.

Ca^{2+}/calmodulin-dependent protein kinase II (CamKII) is a major protein kinase that is capable of regulating the activities of many ion channels and receptors. It is found throughout the central nervous system and it regulates calcium signalling in synaptic transmission. There is strong evidence that CamKII plays a key role in nociceptive processing and sensitization of central sensory neurons. In the superficial layers of the spinal dorsal horns it is abundant both in neuronal perikarya and in the neuropil. The latter is explained by the fact that more than half of dorsal root ganglion cells are CamKII+, especially in the trigeminal ganglion. CamKII immunoreactivity in laminae I–II of the spinal cord is increased after subcutaneous injections of formalin or capsaicin, or after intrathecal injections of substance P, and this increase can be blocked by CamKII inhibitors. After acute noxious stimulation CamKII expression is upregulated in dorsal horn synapses formed by peptidergic primary afferents, which are also crucial in the pathogenesis of headaches.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as acetylsalicylic acid (Aspirin®), are effective in the treatment of acute migraine headache and tension-type headache. This effect could be due to their inhibitory action on cyclooxygenase-2 (COX-2)

and prostaglandins in the spinal trigeminal complex, but they could also inhibit NOS activation by reducing the induction of transcription factor NF kappa ß. Prostaglandins are thought to play role in many neurological functions including the nociceptive processing. Several isoforms of their synthetizing enzyme are known: cyclooxygenase-1 (COX-1) and -2 and the most recently discovered and described cyclooxygenase-3 (COX-3). Recent data suggest that COX-2 may play a critical role in brain development and synaptic signaling and COX-2 expression is upregulated under several pathological conditions. Mainly COX-2 is thought to be responsible for nociceptive processing, but there are also reports suggesting that COX-1 may become over-expressed during inflammation. Constitutively expressed COX-2 is found in the superficial dorsal horn of the rat spinal cord. It has been proposed that prostaglandins synthesized by COX-2 assist in synaptic transmission and enhance postsynaptic activity of both excitatory and inhibitory neurotransmitters. In rats, infusion of COX-1/COX-2 inhibitors blocked sensitization of meningeal nociceptors and suppressed ongoing sensitization in spinal trigeminovascular neurons. A dual-acting COX-2 inhibitor was effective in a rat model of capsaicin-induced central sensitization.

Gonadal steroids, in particular estradiol, modulate nociception and the clinical expression of migraine. After puberty women are three times more affected. Abrupt falls in estrogen plasma levels can trigger the attacks, e.g. in the premenstrual phase, and they may disappear during pregnancy or after menopause, when plasma level of estrogen is stable. In the study of Magos et al. a subcutaneous implant of 17β-estradiol decreased headache intensity of more than 80% of women with menstrual migraine. Moreover, in women undergoing in vitro fertilization, where an analogue of gonadotropin-releasing hormone was administered to downregulate estrogen levels, the low levels of 17β-estradiol correlated with an increased headache prevalence. The neurobiological mechanisms which underlie these modulatory effects of estrogen on migraine remain speculative. Estrogen receptors are present on spinal sensory ganglion neurons and in spinal gray matter. In rats, estrogens are reported to decrease myogenic tone through a NO-dependent mechanism in rat cerebral arteries. In mice it has been shown that estrogens decrease nociception, notably in the trigeminal system. In the rat nitroglycerin model estradiol pretreatment blunts calcitonine gene related peptide (CGRP) and serotonin (5-HT) changes in the superficial laminae of the TNC, which suggest that

ovarian hormones, which greatly influence the course of migraine, have indeed the capacity to modify the expression of pivotal transmitters in the trigeminovascular nociceptive pathway.

On the basis of these data we decided to investigate the possible modulatory effects of COX-inhibition and hormonal influences in the NTG model of migraine.

II. Aims

The aims of our studies were to

i.) study the effect of systemic NTG administration on the CamKII expression of the most caudal part of the TNC of the rat

ii.) examine the possible modulatory effects of estradiol and selective COX inhibitors in the above process.

iii.) determine which isoform of the COX enzyme plays a role in the nNOS activation caused by NTG in the same region.

III. Materials and methods

All experimental procedures described in this paper followed the guidelines of the International Association for the Study of Pain and the European Communities Council (86/609/EEC). They were approved by the Ethics Committee of the Faculty of Medicine, University of Liège and University of Szeged.

III.1. CamKII

III.1.1. Estradiol pretreatment

Systemic NTG and placebo administration (10 mg/kg s.c.) was performed in male, ovariectomised female (ovx) and estradiol treated ovariectomised female (ovx+E) rats. After a delay of 4 hours the cervical part of the TNC was removed and we examined the CamKII expression in its dorsal horns performing immunohistochemistry. The CamKII expression was also quantified with Western blotting.

III.1.2. Selective COX inhibitor pretreatment

Thirty minutes before the NTG and placebo treatment (10 mg/kg s.c.), the first group of rats were injected subcutaneously with the selective COX-2 inhibitor, NS398 at a dose of 1 mg/kg, 3 mg/kg or 5 mg/kg. The second group of rats received a s.c. injection of the selective

COX-1 inhibitor, SC560 at a dose of 1 mg/kg, 5 mg/kg or 10 mg/kg. The third group of animals did not receive any pretreatment. We compared the effect of these drugs on the CamKII induction of NTG in the TNC with immunohistochemistry.

III.2. nNOS

We also compared the effect of the selective COX-2 inhibitor NS398 (at a dose of 1 mg/kg, 3 mg/kg or 5 mg/kg) and the selective COX-1 inhibitor SC560 (at a dose of 1 mg/kg, 5 mg/kg or 10 mg/kg) on the nNOS induction of NTG in the TNC with immunohistochemistry.

IV. Results

IV.1. CamKII

IV.1.1. Estradiol pretreatment

On microscopic examination of immunostained transverse sections, CamKII immunoreactivity was found in neurons of the TNC and in the neuropil of lamina II. As mentioned in the methods, we focused on immunoreactive neurons. CamKII-Ir cells were abundant in the superficial layers of the caudal spinal trigeminal nucleus. The number of cells was not significantly different between the various rostro-caudal levels, nor between sides of the TNC. After vehicle injection there was no significant difference in the number of CamKII-Ir cells in the TNC superficial laminae I-III between male rats, ovariectomized (ovx) and ovariectomized-estradiol treated females (ovx+E2). In contrast, 4 h after subcutaneous NTG administration there was a significant increase in the number of CamKII-Ir cells in males and in ovx animals compared with vehicle injections, but no change was found in ovx + E2 rats.

At the Th1 level the number of CamKII-Ir cells was overall smaller and there was no significant difference between animal groups or between treatment conditions.

The results of Western blotting were in line with those of immunohistochemistry. We identified the bands representing the CamKII protein. In male and ovx animals, which had received NTG 4 h before, the density of the CamKII protein band in the dorsal portion of the C1–C2 segments was increased compared with vehicle-injected rats. In the ovx + E2 group the CamKII band was comparable after NTG or vehicle injection. In the Th1 segments there was no apparent difference in either of the groups.

Densitometric analyses of the blots confirmed that CamKII expression in the dorsal C1–C2 segments was significantly enhanced after NTG administration in male and ovx rats, but not in ovx + E2 animals and that there was no difference between NTG and vehicle injections in the dorsal part of the Th1 spinal segments in either group.

IV.1.2. Selective COX inhibitor pretreatment

During microscopic examinations CamKII-Ir cells were numerous in the superficial layers of the caudal trigeminal nucleus. NTG produced a significant increase of CamKII-positive cells in the superficial layers of the caudal trigeminal nucleus in the non-pretreated rats ($P < 0.05$). This phenomenon was similar when rats received the COX-1 inhibitor, SC560 in various doses (1 mg/kg, 5 mg/kg or 10 mg/kg) before NTG ($P < 0.05$, $P < 0.05$, $P < 0.05$ respectively).

Pretreatment with COX-1 inhibitor in any dosages failed to modulate the NTG-induced CamKII enhancement in the TNC ($P = 1$ for all doses used).

At doses of 3 and 5 mg/kg of the selective COX-2 inhibitor NS398, NTG treatment failed to induce a statistically significant increase in CamKII expression ($P = 0.84$, $P = 0.7$, respectively). The NTG-induced CamKII upregulation was attenuated by 3 mg/kg ($P < 0.05$) and 5 mg/kg ($P < 0.001$) of the selective COX-2 inhibitor.

IV.2. nNOS

Transverse sections of the cervical spinal cord demonstrated many nNOS-Ir neurons in the superficial laminae of the dorsal horns. These cells are mainly small to medium sized neurons (8-15 µm in diameter) with few dentrites. There was no significant difference in the number of Ir cells at different levels of the C1-C2 region. NTG produced significant increase of nNOS-positive cells in the superficial layers of the caudal trigeminal nucleus in the non-pretreated rats ($P < 0.01$).

This phenomenon was similar when rats received the COX-1 inhibitor, SC560 in various doses (1 mg/kg, 5 mg/kg or 10 mg/kg) before NTG ($P < 0.05$, $P < 0.01$, $P < 0.05$ respectively).

The COX-2 inhibitor NS398 given at the dose of 3 mg/kg ($P = 0.82$) or 5 mg/kg ($P = 1$), but not of 1 mg/kg ($P < 0.05$) prior to NTG attenuated the NO-induced nNOS increase. Compared to the control group, after NTG injections, the number of nNOS-Ir neurons was significantly lower in the animals, which received the highest dose of NS398 ($P < 0.05$).

V. Discussion

V.1. NTG-induced CamKII and its modulation by estradiol

Our data demonstrated that systemic administration of NTG enhances CamKII immunoreactivity in laminae I–III of the spinal portion of the TNC. Previous studies have demonstrated increases of c-fos and nNOS expression in TNC after NTG administration at a dose of 10 mg/kg. The latter may be a molecular basis for a self-amplifying process and sensitization, as described clinically during migraine attacks. The effect of the NO donor on nNOS expression seemed to be selective for the trigeminal system, as no effect was detected in upper thoracic segments. We found the same trigeminal selectivity for the NTG-induced CamKII changes. NO probably activates the trigeminal system via an effect on the peripheral nociceptive afferents, since capsaicin pretreatment, which destroys these small afferent fibres, abolishes the NTG-induced c-fos activation of secondary trigeminal nociceptors. NTG itself has different effects on nociception depending on the dose administered. In the rat, an intravenous infusion of glycerylnitrate at low doses (2–50 mg/kg per min) does not cause c-fos activation in the TNC or an increase of jugular vein CGRP levels, despite its capability to enhance stimulus sensitivity in the trigeminal system. In humans, microgram doses are used intravenously or sublingually to trigger migraine attacks. There may thus be species differences in the dose– response relationship of NTG. As far as the time course of the NTG-induced effects is concerned, its brain concentrations and those of cGMP rise significantly 2 h after subcutaneous administration. Fos expression peaks at 1 h in neurons belonging to vasoregulatory areas, but only at 4 h in TNC neurons.

There is strong evidence that CamKII plays a key role in nociceptive processing and sensitization of central sensory neurons. It was shown that CamKII located in the hippocampus is important regulator during the nociceptive processes induced by formalin, glutamate, pro-inflammatory cytokines, and acetic acid injection. Autophosphorylation-deficient CamKII mutant mice display deficiencies in ongoing nociceptive responses in the formalin model, leading to the hypothesis that CamKII is primarily involved in spontaneous nociception. CamKII is abundant in the superficial layers of the dorsal horns, where it is increased during the noxious stimuli induced by capsaicin or by formalin. Intratechal injections of substance P also increases CamKII immunoreactivity in laminae I–II of the spinal cord, and this increase can be blocked by CamKII inhibitors. CamKII protein is

increased in the medullary dorsal horn following nerve injury to the inferior alveolar nerve wherein mechanical allodynia is alleviated by CamKII inhibition. Peripheral CamKII immunoreactivity is also increased following complete Freund's adjuvant -induced inflammation. A study provided evidence that capsaicin stimulates autophosphorylation of CamKII in sensory neurons and that pharmacological inhibition of CamKII reduces vanilloid receptor 1-mediated CGRP release. It was shown that more than half of dorsal root ganglion cells are CamKII-positive, especially in the trigeminal ganglion, where it is expressed in CGRP- and vanilloid receptor 1-immunoreactive neurons. The hyperexcitability induced by capsaicin in trigeminal ganglion neurons via inactivation of I(A) currents is also mediated in part by CamKII, as well as the activation of the vanilloid receptor 1 by phosphorylation in rat ganglion cells. It has been recently discovered that nociceptive stimuli up-regulate CamKII in the dorsal horns by peptidergic afferents, which are also crucial in migraine. In cultured neurons it has been shown that CamKII is able to decrease nNOS activity by phosphorylating this protein, raising the remote possibility that CamKII might counteract the NTG-induced nNOS activation. Moreover, in rats and mice calmodulin can activate various adenylyl cyclases which contribute to sensitization in the spinal cord. In a recent study the CamKII inhibitor, KN93 dose-dependently prevented the inflammation-induced thermal hyperalgesia and mechanical allodynia. We now show that CamKII can also be activated in secondary trigeminal nociceptors by high doses of the NO donor NTG, which suggests that it may play a role in NTG-induced migraine headaches. Although the involvement of CamKII in migraineurs is not yet proven, these data suggest that this enzyme plays an important role in migraine pathogenesis. In our study we demonstrated that systemic administration of NTG enhances CamKII expression in the superficial layers of the TNC, which suggest that CamKII may play a role in sensitization of the trigeminal system and in the pain processes during migraine.

Estrogens are known to modulate nociception, including migraine attacks. It was shown that protracted estrogen deprivation tends to have pronociceptive effects in the orofacial formalin model of pain in mice and in the rat NTG model, where it blunts CGRP and 5-HT changes in the superficial laminae of the TNC. In both instances the observed abnormalities are reversed by estradiol administration. In the hippocampus, where CamKII is known to be crucial for long-term potentiation, estradiol can rapidly induce its neuronal

expression. Our results contrast with the latter, in so far as chronic administration of estradiol has no detectable effect on baseline expression of CamKII in TNC, but is able to suppress its activation by nitroglycerin. Estrogen receptors are present on spinal sensory ganglion neurons and in the spinal grey matter. Estradiol may thus act at the genomic level, which would modulate the expression of CamKII and hence annihilate any detectable change in its immunoreactivity after NTG administration. Whether these findings are relevant to the hormonal influences on migraine remains speculative. However, combined with the clinical observation that female migraineurs are more sensitive to certain trigger factors during the perimenstrual period, they may suggest that the low estrogen levels of the premenstrual and menstrual phases render female migraineurs also more prone to the attack-triggering effects of NTG. By the same token, one could draw a parallel between our data in rats, showing that chronic estradiol treatment suppresses the selective activation of secondary trigeminal nociceptors by nitroglycerin, and the clinical observation that migraine attacks tend to be suppressed when sex hormone levels are high and stable, such as during pregnancy.

In summary, NTG, a NO donor, is able to induce CamKII expression in the superficial layers of the TNC in the rat. This effect is annihilated by chronic high concentrations of estradiol. Considering the known biological properties of CamKII, one may expect that its increased expression after NO enhances nociception in the trigeminal system. In contrast, the suppression of this activation by high estradiol levels can be regarded as trigeminal antinociception. The acute NTG-induced and the chronic estradiol-dependent changes both seem to be selective for the trigeminal sytem. They may thus be relevant to an understanding of the delayed NTG-triggered headache attacks in migraineurs and in patients suffering from chronic tension-type headache, and of the protective effect of stable plasma estrogen levels. They may also give some hints on the molecular effects of NO donors and ovarian steroids in trigeminovascular pain syndromes, such as migraine.

V.2. Effect of COX inhibitors

The major findings of our studies are (1) the selective COX-2 inhibitor NS398 attenuated the NTG-induced nNOS and CamKII activation in the superficial layers of the most caudal portion of the TNC in the rat, (2) in contrast, the pretreatment with a selective COX-1 inhibitor, SC560, failed to modulate this phenomenon.

It is not precisely known how NTG administration modifies the nNOS expression in the TNC. This modification is probably due to a secondary activation of second order nociceptive neurons and/or interneurons by NO excitation of their peripherial afferents, which might initiate a self-amplifying process of NO production possibly leading to central sensitization. Increased prostaglandin E2 release and NO production of monocytes were found in patients with migraine without aura, which indicates that NOS and COX pathways are linked in monocytes. In migraine patients, it has been shown that there is an early activation of the L-arginine/NO pathway and a late rise in the synthesis of prostanoids after the onset of the headache. Since in earlier experiments the nonspecific COX-inhibitor lysine acetyl-salicylate treatment attenuated the NTG-induced nNOS expression, the prostanoids may have an important role in the signal transduction. Since lysine acetyl-salycilate has COX-2 inhibiting property, these earlier data are concordant with our present observation about the effect of the selective COX-2 inhibitor NS398 on the NTG-induced nNOS activation. Our study provides the first evidence that the elevation in nNOS immunoreactivity can be influenced selectively by COX inhibitors.

Furthermore we have shown that the selective COX-2 inhibitor, NS398 attenuated the NTG-induced increase of CamKII expression in the superficial layers of the lower TNC in rats. In contrast, pre-treatment with a selective COX-1 inhibitor, SC560 was not able to modulate this phenomenon. COX-1 can be found in the sensory ganglia and the spinal cord and there are results suggesting its involvement in nociception. Previous studies have demonstrated that oral SC-560 at a dose of 30 mg/kg can reduce carragenan-induced thermal hyperalgesia. It is also known that SC-560 displays low (<15%) bioavailability after given orally. Comparable parenteral dosage of SC-560 in our study failed to modulate the NTG induced increase of CamKII expression suggesting that COX-1 is not involved in this phenomenon.

It has been shown that systemic NTG increased the expression of COX-2 and prostaglandin E2 in the lower brain stem after 4 h. COX-2 inhibitors are effective in the treatment of migraine. In animals, constitutively expressed COX-2 is found in the superficial dorsal horn of the rat spinal cord. It has been proposed that prostaglandins, synthesized by COX-2, assist in synaptic transmission and enhance postsynaptic activity of both excitatory and inhibitory neurotransmitters. These data may explain the fact that COX-2- and/or COX-2-

derived metabolites play an important role in central sensitization and they are thought to mediate most of the analgesic effects of NSAIDs. In rats, nimesulide, a preferential COX-2 inhibitor, showed a significant analgesic effect in tailflick and in formalin tests, and it was also effective in these tests after NTG-induced hyperalgesia. Contrary to the COX-2 inhibitor celecoxib, the COX-1 inhibitor SC560 failed to reduce edema and hyperalgesia after carrageenan induced inflammation in the rat. Oral and intrathecal administration of SC560 did not alter the behavior changes in the formalin test where celecoxib and indomethacin were effective.

Taken together these data and our present results suggest that the stimulating effect of NTG and that of NO on nNOS and CamKII expression in secondary trigeminal nociceptors are mediated by COX-2-expressing interneurons in the TNC superficial laminae. Therefore, COX-2 may participate in the activation of the trigeminal system and may be a crucial enzyme in the pathophysiology of headaches. We have demonstrated that NO-induced nNOS and CamKII expression in the TNC of the rat is dose-dependently attenuated by pretreatment with COX-2 but not with COX-1 inhibitors. These data suggest that prostanoids, especially produced via the COX-2 pathway, are involved in NO-mediated activation of the trigeminal system. The fact that COX-2 plays a role in this self-amplifying process of the trigeminal area may give us further details about headache and migraine pathophysiology.

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