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Central Components of the Analgesic/ Antihyperalgesic Effect of Nimesulide: Studies in Animal Models of Pain and Hyperalgesia

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Abstract

The analgesic action of NSAIDs has been attributed to the peripheral inhibition of prostaglandin synthesis via the blockade of the enzyme cyclo-oxygenase (COX) and prevention of bradykinin and cytokine-induced hyperalgesia via inhibition of the release of tumour necrosis factor-α. However, it is becoming increasingly evident that NSAIDs exert their analgesic effect through several mechanisms. Recent data suggest that significant expression of COX-2 is found in the central nervous system, where COX-2 seems to have, together with nitric oxide, an important role in spinal nociceptive transmission. Nitroglycerin is a nitric oxide donor and induces a hyperalgesic state, partially mediated by central mechanisms. Nimesulide is a preferential COX-2 inhibitor widely used to treat pain.

In this study, we evaluated the analgesic effect of nimesulide in several animal models of pain, intending to provide additional information on the characteristics of the analgesic effect of nimesulide, with specific focus on a possible central component.

Study Design: Nimesulide was compared with vehicle in groups of 4–10 rats that were randomly tested with different models of pain. The experimental design also included study of the effect of nimesulide upon nitroglycerin-induced neuronal activation at central sites. Analysis of variance was used to evaluate the influence of time and treatments. Differences between groups at specific time-points were analysed by *post-hoc* t-test. A probability level of less than 5% was regarded as significant.

Methods: The analgesic effect of nimesulide (or vehicle) was evaluated in male Sprague-Dawley rats. The animals underwent tail-flick and formalin tests, both performed in baseline conditions and after nitroglycerin-induced hyperalgesia. Two separate groups of rats were treated with nitroglycerin alone or nimesulide followed by nitroglycerin, and their brains were processed for immunocytochemical detection of Fos protein, a marker of neuronal activation.

Results: Nimesulide showed a significant analgesic effect in both the tail-flick and the formalin tests in baseline conditions. In addition, the drug proved effective in counteracting nitroglycerin-induced hyperalgesia in both tests. Brain mapping of

nuclei activated by the administration of nitroglycerin showed that nimesulide pretreatment significantly inhibited neuronal activation in several areas, namely the supraoptic nucleus, ventrolateral column of the periaqueductal grey, locus coeruleus, nucleus tractus solitarius and area postrema.

We conclude that nimesulide possesses a strong analgesic and antihyperalgesic activity, the mechanisms of action of which are partly central.

Introduction

Synthesis of prostaglandin (PG) by cyclo-oxygenase (COX) has long been associated with inflammatory diseases and pain.[1] PGs are involved in hyperalgesia[2] and play an important part in the transmission of nociceptive information at the spinal level.[3-7] They are derived from both non-neuronal and neuronal pools by different enzymatic isoforms.[8,9] Generally, a constitutively active COX-1 generates the physiological pools of PGs whereas, on appropriate stimulation, an inducible COX-2 generates pathological quantities of prostanoids. However, it has been shown that COX-2 may have a constitutive activity in neurons, where its functional status can be modulated by synaptic events. The COX-2 isoform predominates in the spinal cord, where it concentrates in the regions implicated in the reception of nociceptive stimuli.

Nitric oxide (NO) is an important modulator of neuronal activity, which mediates the processing of nociceptive information in the central nervous system (CNS).[10,11] Several studies have suggested the involvement of NO in the development and maintenance of hyperalgesia.[12-18] NO can exert its effects both intracellularly, at its site of origin, and intercellularly,[19,20] and it may facilitate nociception either with an action upon intrinsic dorsal horn neurons, transmitting nociceptive information to cerebral centres, or retrogradely, via diffusion from sensitised dorsal horn neurons to presynaptic terminals.[21-23]

A delicate interplay exists between PGs and NO, as is suggested by the demonstration that NO promotes COX activity and the release of PGs provokes a further feedback increase in production of NO.[24,25]

The analgesic action of NSAIDs has been explained on the basis of the peripheral inhibition of PG synthesis via the blockade of COX and prevention of bradykinin- and cytokine-induced hyperalgesia via inhibition of the release of tumour necrosis factor-α.[26] However, it is becoming increasingly evident that NSAIDs exert their analgesic effect through a variety of other peripheral and central mechanisms. These latter are represented by interaction with endogenous opioid peptides and release of serotonin,[27,28] and the activation of the *N*-methyl-*d*-aspartate (NMDA) receptor.[29] The localisation of COX-2 in the brain[30] provides an additional central mechanism of action for several NSAIDs, in particular for those that have a preferential inhibitory effect upon COX-2, such as nimesulide.[31]

The most traditional tests of nociception for evaluating the analgesic effect of drugs are represented by the tail-flick, hot-plate and formalin tests.[2] Tail-flick and hot-plate tests simply reflect the activation of nociceptive pathways by a physiological, phasic stimulus of high intensity, whereas the formalin test allows the evaluation of nociceptive behaviour in response to tonic pain generated by injured tissue, with the mediation of spinal mechanisms.

Previous studies by our group have shown that nitroglycerin, a NO donor, activates specific nociceptive nuclei in the rat,[32-35] via the induction of a hyperalgesic state, as confirmed by recent studies that demonstrated the ability of nitroglycerin to increase the rate of discharge of spinal nociceptive neurons,[36] to activate the transcription factor, NF-κB,[37] and to induce hyperalgesic responses in the tail-flick and formalin tests in rats.[38]

The objective of the present study was to investigate further the analgesic effect of nimesulide in different animal models of pain. Particular

attention was devoted to the possible efficacy of the drug in hyperalgesic conditions and to the identification of its putative site(s) of action.

Study Design

Nimesulide was compared with vehicle in groups of 4–10 rats that were randomly tested with different models of pain: the tail-flick test and the formalin test. Both tests were performed in untreated animals and in rats made hyperalgesic by means of the preventative systemic administration of nitroglycerin.[31] The experimental design also included study of the effect of nimesulide on nitroglycerin-induced neuronal activation at central sites.

Methods

Drugs

Nimesulide (Helsinn Healthcare SA, Switzerland), suspended in saline, was injected intraperitoneally at a dose of 2.5 mg/kg.

Nitroglycerin (Astra Company, Italy), dissolved in saline alcohol and propylene glycol, was injected intraperitoneally at a dose of 10 mg/kg.[32,33,38]

For the formalin test, a 100μL volume of 1% formalin (formaldehyde diluted in 0.9% saline) was injected by the intraplantar route.

Tail-Flick Test

The tail-flick test consists of recording the latency of reflex withdrawal of the tail from a high-intensity light beam, a measure of physiological phasic pain.

The test was performed with a Tail Flick instrument (model 7360, Ugo, Basile) that allowed automatic recording of the latency of the tail-flick response to radiant heat.[39] Latency at each evaluation was calculated as the mean of three measurements in three different parts of the tail. A cut-off limit of exposure corresponding to 20s was set, to prevent tissue damage.

Each animal was placed on the recording platform of the instrument, where it was kept under slight, painless restraint, with its tail positioned on the radiant heat window.

Formalin Test

For this test, one animal at a time was placed into a plexiglas observation chamber (10 × 20 × 24cm) with a mirror (45° angle) positioned to permit unhindered observation of the animal's paw. Formalin was injected subcutaneously into the centre of the plantar surface of the left hind paw, with slight restraint. A 26-gauge needle connected to a 1mL syringe was used and the solution was delivered as rapidly as possible while the animal was immobilised. The rat was then replaced in the box, the clock was started and the pain response was recorded for a period of 1 hour.[40]

Pain-related behaviour was quantified for 1 hour by counting spontaneous flinches and shakes of the injected paw: over 60-second periods for the first 5 minutes (minutes 1, 2, 3, 4 and 5) and thereafter following 4-minute pauses, for 1-minute periods up to the hour. Phase I was defined as the period from 1 to 5 minutes; phase II was defined as the period from 10 to 60 minutes inclusive.[41] Phase I is generally considered to represent the chemical activation of nociceptors, whereas phase II reflects the inflammatory reaction and central processing.

Immunocytochemical Detection of Fos

The effect of nimesulide on nitroglycerin-induced neuronal activation was investigated by means of the immunocytochemical detection of expression of Fos protein, a marker of neuronal activation.

At the beginning of this experiment, the general behaviour of each rat was observed and recorded, for a 4-hour period after the administration of drugs, by an observer blinded to the treatments.

Animals were anaesthetised and perfused transcardially with saline and ice-cold 4% paraformaldehyde 4 hours after the administration of the drug. Brains were removed, post-fixed for 12 hours in the same fixative and subsequently transferred into

solutions of sucrose at increasing concentrations (up to 30%) over the following 72 hours. Brains were cut at 50μm on a freezing sliding microtome. Expression of Fos in the rat brain was detected by means of the immunohistochemical technique with a rabbit polyclonal antiserum directed against Fos protein (residues 4–17 of human Fos). Tissue sections were incubated for 48 hours at 4°C with the Fos antibody (Oncogene). After thorough rinsing in buffer, sections were processed with the avidin-biotin technique, using a commercial kit. Cells positively stained for Fos were visualised with nickel-intensified 3′,3′-diaminobenzidine tetrahydrochloride. After staining, sections were rinsed in buffer, mounted onto glass slides, air-dried and coverslipped.

Statistical Evaluation

Analysis of variance was used to evaluate the influence of time and treatments. Differences between groups at specific time-points were analysed by *post-hoc* t-test. A probability level of less than 5% was regarded as significant.

Experimental Groups

Adult male Sprague-Dawley rats (weight 180–220g) were evaluated in these experiments. Experiments conformed to the Guidelines of the European Union and to guidelines of the International American Society for Study of Pain (IASP) for pain research in animals. Rats were housed in plastic boxes in groups of three, with water and food available *ad libitum*, and kept on a 12/12 hours light/dark cycle. All animals were acclimatised to the test chamber before testing.

Rats were randomly divided into groups comprised of 4–10 animals each, and underwent the following experimental procedures.

Tail-Flick Test

The experimental plan consisted of the following treatment groups:

Group 1: evaluation at baseline and 2 hours after nimesulide administration;
Group 2: evaluation at baseline, and 2 and 4 hours after nitroglycerin injection;
Group 3: evaluation at baseline. Rats were then injected with nimesulide followed, 30 minutes later, by nitroglycerin. The tail-flick test was repeated 2 and 4 hours after nitroglycerin administration;
Group 4: evaluation at baseline. Rats were then injected with nitroglycerin followed, 2 hours later, by nimesulide. The tail-flick test was evaluated 4 hours after nitroglycerin administration.

Formalin Test

The experimental plan consisted of the following treatment groups:
Control group: test 2 hours after saline;
Nime group: test 2 hours after nimesulide;
NTG 2h group: test 2 hours after nitroglycerin;
NTG 4h group: test 4 hours after nitroglycerin;
Nime + NTG 2h group: nimesulide at −30 minutes, nitroglycerin at T0 and formalin test 2 hours later;
Nime + NTG 4h group: nimesulide at −30 minutes, nitroglycerin at T0 and formalin test 4 hours later.

Fos Expression

Two groups of animals were evaluated according to the following schedule:
NTG group (n = 8): rats received nitroglycerin and were perfused 4 hours later (previously identified as the time of maximal nitroglycerin-induced neuronal activation[32-35]);
Nime + NTG group (n = 10): rats received nimesulide at −30 minutes, nitroglycerin at T0 and were perfused 4 hours later.

Results

Tail-Flick Test

Nimesulide significantly increased the latency

of the tail-flick response (group 1) [figure 1]. Conversely, nitroglycerin induced a hyperalgesic response, as suggested by the significant decrease in the latency of the tail-flick response. This hyperalgesic response was observed 2 and 4 hours after the administration of nitroglycerin (group 2) ($p = 0.046$ and 0.028, respectively) [figure 2].

Pretreatment with nimesulide (group 3) blocked nitroglycerin-induced hyperalgesia 2 hours, but not 4 hours, after the administration of nitroglycerin (figure 3a). However, a significant block of nitroglycerin-induced hyperalgesia was observed also at 4 hours after nitroglycerin administration when nimesulide was injected 2 hours *after* nitroglycerin (group 4) [figure 3b].

No statistically significant differences were observed between group 1 (nimesulide) and group 3 (nimesulide + nitroglycerin), whereas the analgesic effect of nimesulide was significantly less marked in group 4 (nitroglycerin + nimesulide) than in group 1 ($p < 0.03$).

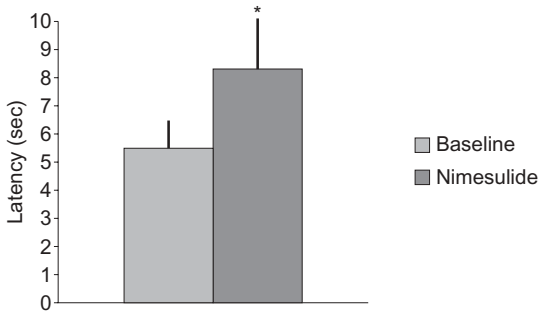

Fig. 1. Histogram illustrating the antinociceptive effect of nimesulide in the tail-flick test, as suggested by the significant increase in the latency of the response recorded 2 hours after its administration. * $p < 0.03$ vs baseline.

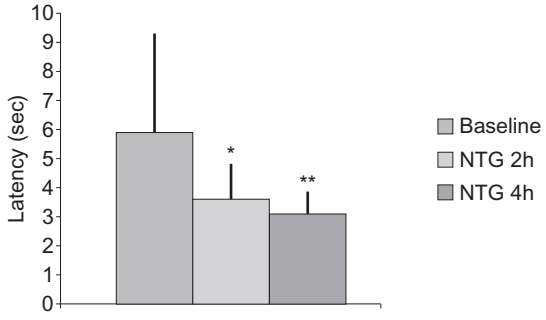

Fig. 2. Histogram illustrating the hyperalgesic effect of nitroglycerin (NTG) in the tail-flick test, as suggested by the significant reduction in latency of the tail response observed at 2 and 4 hours. * $p < 0.05$, ** $p < 0.03$ vs baseline.

Formalin Test

In the control group, injection of formalin resulted in a highly reliable, typical, biphasic display of flinches and shakes of the injected paw, with an early phase (phase I) of nociception occurring within the first 5 minutes, and a late phase (phase II) extending from 15 to 60 minutes after formalin injection. Administration of nimesulide (Nime group) induced a significant reduction in the total number of flinches/shakes in both phases as compared with the control group (figure 4).

Nitroglycerin induced a hyperalgesic response to the formalin test, as demonstrated by the significant increase in the number of flinches/shakes observed 2 and 4 hours after its administration (figure 5). Rats in the NTG 2h group showed a significant increase in the total number of flinches/shakes in both phases of formalin test, whereas rats in the NTG 4h group showed a statistically significant increase in the number of flinches/shakes that was limited to phase II. In both groups, the increase in the total number of flinches/shakes was located within the 30-to-50-minute interval.

Pretreatment with nimesulide significantly decreased the total number of flinches/shakes in both phases of the formalin test in rats tested 2 hours after the administration of nitroglycerin (Nime + NTG 2h group) and during phase II in rats tested 4 hours after nitroglycerin administration (Nime + NTG 4h group) [figure 6].

No significant differences were observed when phase I nociceptive behaviour was compared

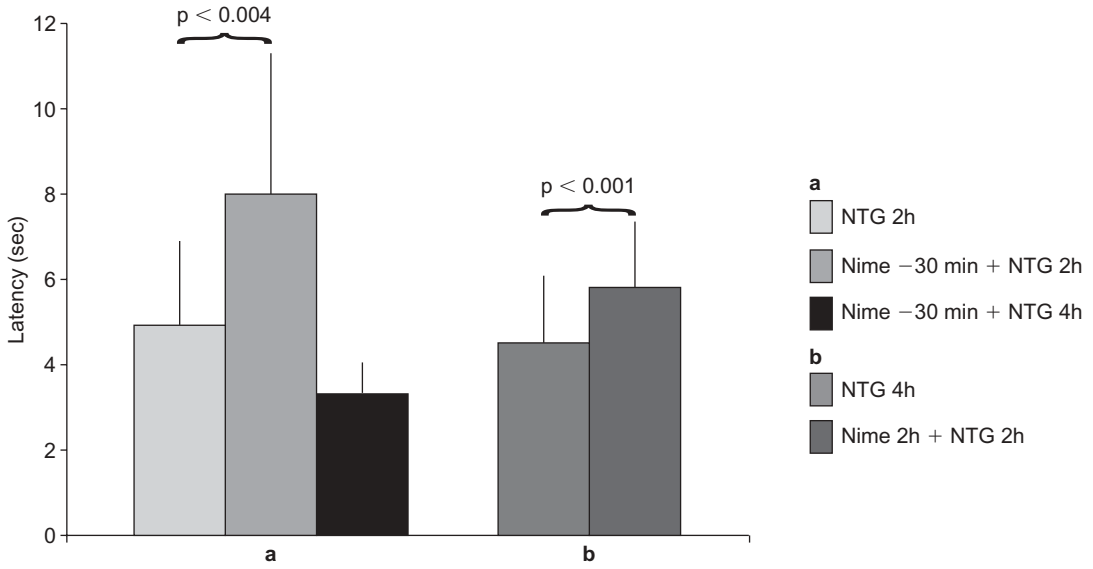

Fig. 3. Nimesulide (Nime) reversed the hyperalgesia induced by nitroglycerin (NTG) at 2 hours when administered preventatively (**a**). The antinociceptive effect of nimesulide upon NTG-induced hyperalgesia was observed at 4 hours only when nimesulide was administered 2 hours after the nitroderivative (**b**).

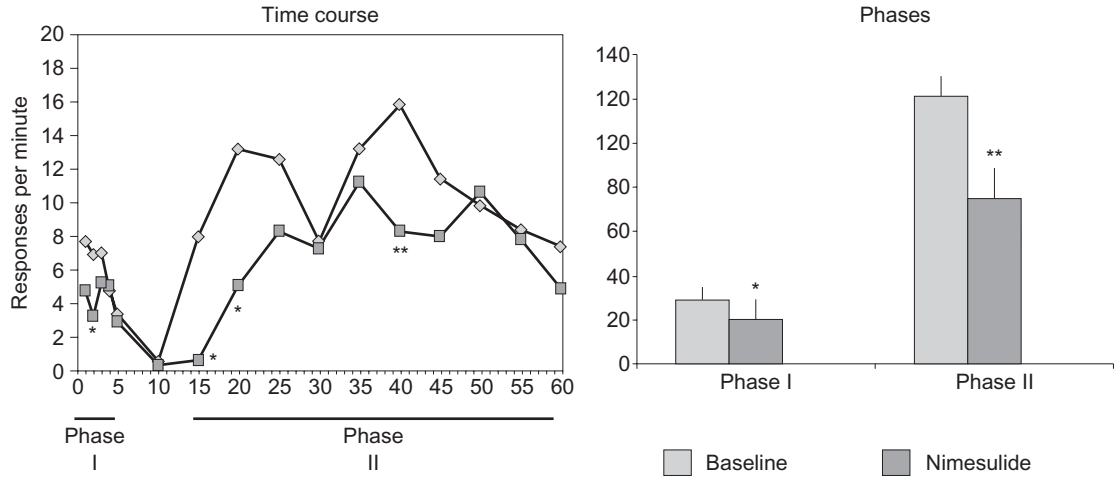

Fig. 4. Nimesulide induced a significant antinociceptive effect (reduction in the number of flinches/shakes of the paw per minute) in both phases of the formalin test compared with vehicle (controls). * $p < 0.05$, ** $p < 0.0001$ vs control.

between the Nime group and the Nime + NTG 2h group or Nime + NTG 4h group. When phase II nociceptive behaviour was analysed, no significant differences were observed between Nime and Nime + NTG 4h groups, but the reduction in formalin-related nociceptive behaviour was less marked in the Nime + NTG 2h group: the number of fliches/shakes during phase II was 112.2. ± 20 in the Nime + NTG 2h group and 75 ± 16.7 in the Nime group ($p < 0.006$).

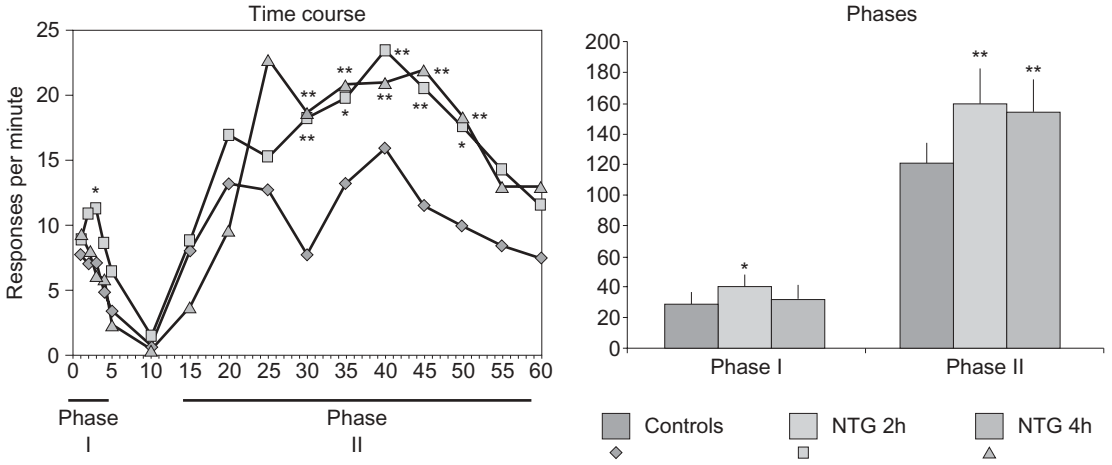

Fig. 5. Nitroglycerin (NTG) induced an increase in nociceptive behaviour (number of flinches/shakes of the paw per minute) at 2 and 4 hours compared with vehicle (controls). This phenomenon was more consistent and marked during phase II. * $p < 0.05$, ** $p < 0.02$ vs control.

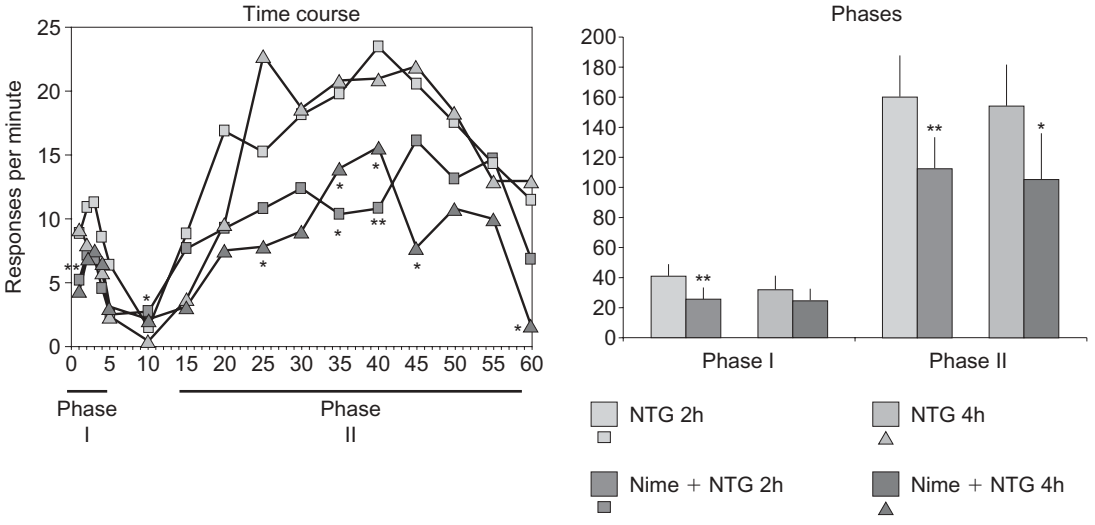

Fig. 6. Nimesulide (Nime) induced a decrease in formalin-evoked nociceptive behaviour at 2 and 4 hours after the administration of nitroglycerin (NTG). * $p < 0.05$, ** $p < 0.02$ vs NTG.

Fos Expression

In agreement with our previous findings,[31,32] administration of nitroglycerin activated brain nuclei located in the prosencephalon (paraventricular and supraoptic nuclei of the hypothalamus, central nucleus of the amygdala) and in the brainstem (ventrolateral column of the periaque-

ductal grey, parabrachial nucleus, locus coeruleus, nucleus tractus solitarius, area postrema and nucleus trigeminalis caudalis) [figure 7].

Nimesulide pretreatment significantly reduced nitroglycerin-induced neuronal activation in many of these nuclei, achieving a statistically significant effect in the supraoptic nucleus, ventrolateral column of the periaqueductal grey, locus coeruleus, nucleus tractus solitarius and area postrema (figures 7 and 8).

Behaviour

In accordance with our previous findings, administration of nitroglycerin induced a stereotyped behaviour that consisted of an almost complete inactivity, without eating and drinking, for the entire period of observation.[32] Rats treated with nimesulide alone behaved normally. The rats pretreated with nimesulide before receiving nitroglycerin were only slightly hypoactive when compared with both the control group and the nimesulide-alone group.

Discussion

Chronic pain states are mediated by peripheral and central factors. Tissue injury leads to a constellation of changes in spinal excitability, which include increased spontaneous firing, increased response amplitude and duration, de-

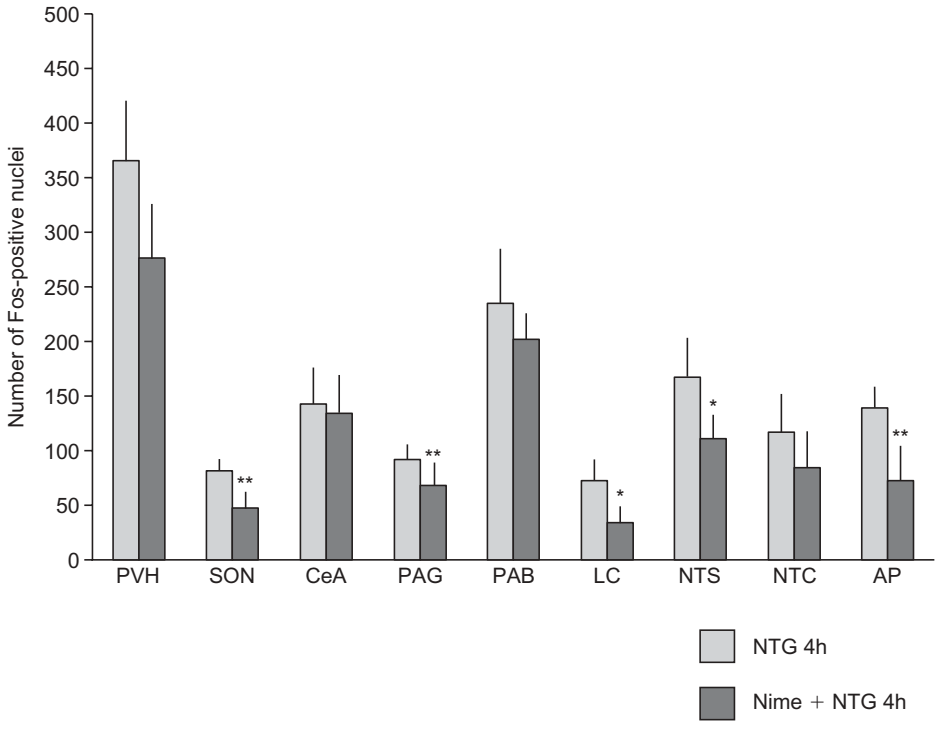

Fig. 7. Pretreatment with nimesulide induced a significant inhibition of nitroglycerin-induced neuronal activation in several brain nuclei, including the supraoptic nucleus (SON), periaqueductal grey (PAG), locus coeruleus (LC), nucleus tractus solitarius (NTS) and area postrema (AP). The effects in the paraventricular nucleus of the hypothalamus (PVH), the central nucleus of the amygdala (CeA), the parabrachial nuclues (PAB) and the nucleus trigeminalis caudalis (NTC) did not achieve statistical significance.
* $p < 0.03$, ** $p < 0.01$ vs nitroglycerin (NTG) 4h.

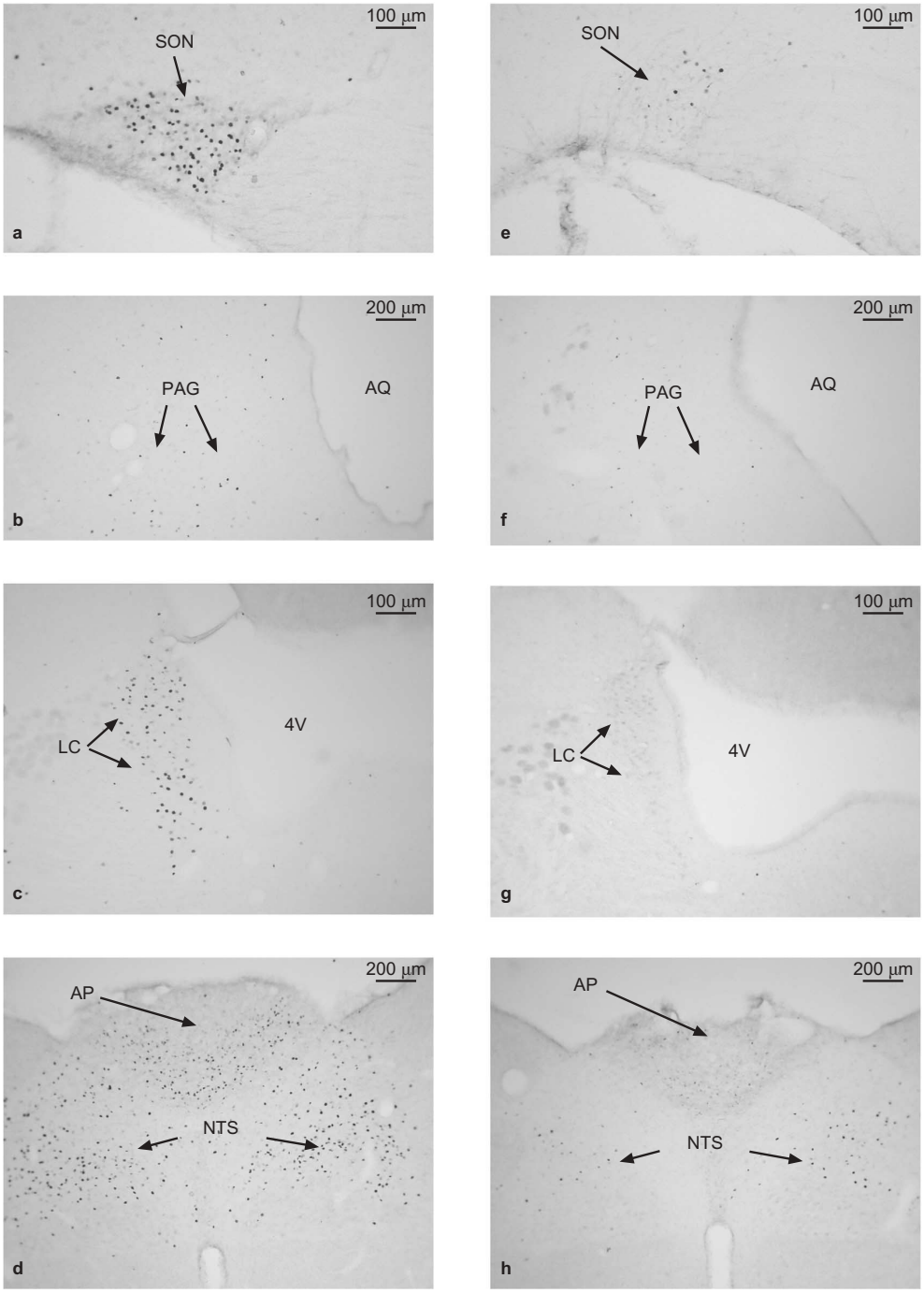

Fig. 8. Micrographs of representative sections of the supraoptic nucleus (SON), ventrolateral column of the periaqueductal grey (PAG), locus coeruleus (LC), nucleus tractus solitarius (NTS) and area postrema (AP) of rats treated with nitroglycerin (**a–d**) and of rats pretreated with nimesulide before receiving nitroglycerin (**e–h**). **AQ** = aqueduct; **4V** = fourth ventricle.

creased threshold, enhanced after discharge to repeated stimuli, and expanded receptive fields.[42] The persistence of these changes, which are collectively termed 'central sensitisation', appears to be fundamental for the prolonged enhancement of pain sensitivity and might explain why patients perceive non-nociceptive stimuli as painful (allodynia) or experience abnormally large responses to normal nociceptive stimuli (hyperalgesia).

Nimesulide is an NSAID, widely used to treat chronic pain and inflammation. Clinical studies have established its analgesic, anti-inflammatory and antipyretic effectiveness.[31] The analgesic effect of the drug has been proved also in experimental animal models of pain. The few studies that have compared the analgesic potency of nimesulide with other NSAIDs have yielded conflicting results. With the tail-flick test, nimesulide has proved both equally[43] and more[44] effective than diclofenac. With the writhing test, it has proved both more[44] and less[45] potent than diclofenac. In two models of thermal and mechanical hyperalgesia, nimesulide was more potent than diclofenac and celecoxib.[46] These disparities may be reflections of various methodological details, including route of administration, stimulus intensity and dosing interval. An important issue is undoubtedly related to the fact that, when studying analgesic drugs, it is of outstanding importance to distinguish between physiological (nociceptive) pain and hyperalgesia, a substantial component of which is generated within the spinal cord.

In the present study, we devised a complex procedure that allowed us to investigate the antinociceptive activity of nimesulide on different modalities of pain (thermal, chemical, inflammatory) in baseline conditions and during hyperalgesia.

Our findings confirm the antinociceptive activity of the drug after intraperitoneal administration.[44,46,47] A significant analgesic effect was observed in both the tail-flick test, which evaluates phasic and physiological pain, and the formalin test, which evaluates tonic and inflammatory pain. In addition, nimesulide was effective at counteracting nitroglycerin-induced hyperalgesia in both tests. Nitroglycerin-induced hyperalgesia was detected 2 and 4 hours after the drug administration. Pharmacokinetic studies show that nitroglycerin has a very short half-life in plasma and peripheral tissue, whereas it accumulates in the brain, where it reaches maximal concentrations 2 hours after its administration.[48] Intradermal administration of nitroglycerin does not alter the thermal pain threshold in humans,[49] but reduction of NO at the peripheral level blocks the antinociceptive effect of nimesulide.[47] Taken together, these findings suggest that nitroglycerin-induced hyperalgesia is mediated by an increased availability of NO at central sites, rather than in the periphery. Therefore, the findings regarding the effect of nimesulide on nitroglycerin-induced hyperalgesia strongly suggest that the mechanism of action of this NSAID is, at least partly, related to central mechanisms. This speculation is further supported by the data obtained in the tail-flick test: the antihyperalgesic activity of nimesulide was also observed when it was administered 2 hours after nitroglycerin (i.e. when the increased availability of NO at the peripheral level had disappeared) [figure 3b]. In this regard, it is noteworthy that the antihyperalgesic effect of nimesulide was greatest when the drug was administered before nitroglycerin, which suggests that preventive COX-2 inactivation is necessary to achieve the most effective block of NO-related thermal hyperalgesia.

The findings obtained with the formalin test further suggest the role of central mechanisms in the action of nimesulide. In agreement with previous reports,[43,47] the analgesic/antihyperalgesic effect of nimesulide extended over both phases of the test, being more marked during phase II. Phase II of the formalin test represents a prolonged tonic response in which inflammatory processes are involved and neurons in the dorsal horns of spinal cord are activated.[39] It is noteworthy that nimesulide completely prevented the nitroglycerin-related potentiation of nociceptive behaviour in the formalin test 4 hours after nitroglycerin, whereas the inhibition was only

partial at 2 hours. Conversely, nimesulide was more effective at the earlier time-point (2 hours) when tested in the tail-flick test. Taken together, these findings suggest that the analgesic and antihyperalgesic effects of nimesulide are probably related to reduction of pain transmission at the spinal level. The present findings are in agreement and expand on previous human data regarding the inhibitory effect of nimesulide upon the spinal nociceptive flexion reflex before and after the administration of nitroglycerin to healthy individuals.[50]

A central effect for nimesulide is also supported by its physicochemical characteristics – a relatively high pKa (approximately 6.5) and a moderate lipophilicity – which support an easy diffusion to the brain. Orally administered nimesulide results in a brain concentration of approximately 1 μg eq./g 3 hours after administration.[51] This tissue concentration corresponds to those that induce inhibition of activity of COX-2.[52,53]

The inhibition of nitroglycerin-induced hyperalgesia was paralleled by the inhibition of nitroglycerin-induced neuronal activation in specific brain nuclei involved in nociceptive and autonomic control. The ventrolateral column of the periaqueductal grey has an important role in the control of nociception and in the coupling of pain perception with autonomic responses. The nucleus tractus solitarius, area postrema and supraoptic nucleus are deeply involved in the control of autonomic function. The locus coeruleus is pivotal in the integration of autonomic and nociceptive function (reviewed by Tassorelli and Joseph[33]). Surprisingly, no significant inhibition of nitroglycerin-induced expression of Fos was observed in the nucleus trigeminalis caudalis, a nucleus with a primary nociceptive function, which has been reported to be inhibited by indomethacin.[34] Taken together, these data on the effect of nimesulide on nitroglycerin-induced activation of Fos in the central nervous system support a role for supraspinal mechanisms in the analgesic effect of nimesulide. The possible location of sites in which these mechanisms take place is suggested by our findings, whereas their mediators remain elusive. With the exception of the supraoptic nucleus, all the brain nuclei inhibited by nimesulide pretreatment receive a rich serotonergic innervation, which suggests that nimesulide may, at least partly, owe its analgesic effect to the interaction with the central serotonergic system, analogous to what has been demonstrated for other simple analgesics.[27,28]

Identification of the mediators involved in the effect of nimesulide was beyond the scope of the present investigation. Controversies exist as to the relative role of the two COX isoforms in the mediation of central nociception. Both COX isoforms are constitutively expressed in spinal cord,[54,55] and they contribute to the nociception-induced increase in PGE_2 that is associated with nociceptive behaviour. Selective COX-2 inhibitors are poorly effective or ineffective in reducing formalin-induced nociceptive behaviour, whereas non-selective COX inhibitors and selective COX-1 inhibitors significantly produce antinociception that is limited to the second phase of the test.[56-59] Therefore, one would argue that COX-1 inhibition is more relevant for preventing formalin-induced nociceptive behaviour. In contrast, the application of sophisticated procedures for evaluating pain and central hyperalgesia allowed Yaksh et al. to show that constitutive COX-2, but not COX-1, contributes to spinally mediated hyperalgesia.[60] Our findings demonstrate that a preferential inhibitor of COX-2 may have advantages over both selective inhibitors of COX-1 and selective inhibitors of COX-2 in condition of acute inflammatory pain and nitric oxide-mediated hyperalgesia.

In addition to COX-2 inhibition,[61] other possible mediators of the effect of nimesulide are excitatory amino acids and peptides, such as glutamate and substance P, which are released from primary afferents and dorsal horn neurons.[62-66] NO and PGs increase the release of glutamate[67,68] that provokes sensitivity of dorsal horn neurons. Spinal hyperalgesia induced by NO donors and PG_2 may be blocked by NMDA receptor antagonists.[69,70]

In future work, it will be of considerable interest

to determine the functional role that is played by the above-mentioned mediators in the antihyperalgesic activity of nimesulide.

In summary, nimesulide showed consistent antinociceptive activity in different rodent models of nociceptive pain and hyperalgesia analgesic. This is likely to depend, at least partly, upon a direct action of the drug on spinal and supraspinal structures.

References

1. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol 1971; 231: 232-5
2. Ito S, Okuda-Ashitaka E, Minami T. Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. Neurosci Res 2001; 41: 299-332.
3. Taiwo YO, Levine JD. Prostaglandins inhibit endogenous pain control mechanisms by blocking transmission at spinal noradrenergic synapses. J Neurosci 1998; 8: 1346-9
4. Uda R, Horiguchi S, Ito S, et al. Nociceptive effects induced by intrathecal administration of prostaglandin D_2, E_2, F_2 to conscious mice. Brain Res 1990; 510: 26-32
5. Malmberg AB, Yaksh TL. Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. Science 1992; 257: 1276-9
6. Minami T, Nishihara I, Uda R, et al. Characterization of EP-receptor subtypes involved in allodynia induced by intrathecal administration of prostaglandin E_2. Br J Pharmacol 1994; 112: 735-40
7. Minami T, Uda R, Horiguchi S. Allodynia evoked by intrathecal administration of prostaglandin E_2. Pain 1994; 57: 217-23
8. Vanegas H, Schaible H-G. Prostaglandins and cyclooxygenases in the spinal cord. Prog Neurobiol 2001; 64: 327-63
9. Millan MJ. The induction of pain: an integrative review. Prog Neurobiol 1999; 57: 1-164
10. Kawabata A, Umeda N, Takagi H. L-Arginine exerts a dual role in nociceptive processing in the brain: involvement of the kyotorphin-Met-enkephalin pathway and NO-cyclic GMP pathway. Br J Pharmacol 1993; 109: 73-9
11. Malmberg AB, Yaksh TL. Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. Pain 1993; 54: 291-300
12. Wiertelak EP, Furness LE, Watkins LR, et al. Illness-induced hyperalgesia is mediated by a spinal NMDA-nitric oxide cascade. Brain Res 1994; 664: 9-16
13. Linden DR, Seybold VS. Spinal neurokinin receptors mediate thermal but not mechanical hyperalgesia via nitric oxide. Pain 2000; 80: 309-17
14. Hamalainen MM, Lovick TA. Involvement of nitric oxide and serotonin in modulation of antinociception and pressor response evoked by stimulation in the dorsolateral region of the periaqueductal gray matter in the rat. Neuroscience 1997; 80: 821-7
15. Aley KO, Mc Cater G, Levine JO. Nitric oxide signaling in pain and nociceptor sensitization in the rat. J Neurosci 1998; 18: 7008-14
16. Gao WC, Ojao JT. Nitric oxide contributes to both spinal nociceptive transmission and its descending inhibition in rats: an immunocytochemical study. Neurosci Lett 1998; 40: 143-6
17. Ferreira J, Santos AR, Calixtom JB. The role of systemic spinal and supraspinal L-arginine-nitric oxide-cGMP pathway in thermal hyperalgesia caused by intrathecal injection of glutamate in mice. Neuropharmacology 1999; 38: 835-42
18. Urban MO, Gebhart GF. Supraspinal contribution to hyperalgesia. PNAS 1999; 96: 7687-92
19. Dawson TM, Snyder SH. Gases as biological messengers: nitric oxide and carbon monoxide in the brain. J Neurosci 1994; 14: 5137-53
20. Garthwaite J, Boulton CL. Nitric oxide signaling in the central nervous system. Annu Rev Physiol 1995; 57: 683-706
21. Coderre TJ. The role of excitatory amino acid receptors and intracellular messengers in persistent nociception after tissue injury in rat. Molec Biol 1993; 7: 229-46
22. Coderre TJ, Yashpal K. Intracellular messengers contributing to persistent nociception and hyperalgesia induced by L-glutamate and substance P in the rat formalin pain model. Eur J Neurosci 1994; 6: 1328-34
23. Kitto KF, Haley JE, Wilcox GL. Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. Neurosci Lett 1992; 148: 1-5
24. Bezzi P, Carmignoto G, Pasti L, et al. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 1998; 391: 281-5
25. McCormack K. Non-steroidal anti-inflammatory drugs and spinal nociceptive processing. Pain 1994; 59: 9-43
26. Ferreira SH. The role of interleukins and nitric oxide in the mediation of inflammatory pain and its control by peripheral analgesics. Drugs 1993; 46: 1-9
27. Kaube H, Hoskin HL, Goadsby PJ. Intravenous acetylsalicylic acid inhibits central trigeminal neurons in the dorsal horn of the upper cervical spinal cord in the cat. Headache 1993; 33: 541-4
28. Sandrini M, Vitale G, Pini LA. Central antinociceptive activity of acetylsalicylic acid is modulated by brain serotonin receptor subtypes. Pharmacology 2002; 65: 193-7
29. Cashman JN. The mechanisms of action of NSAIDs in analgesia. Drugs 1996; 52 Suppl. 5: 13-23
30. O'Neill GP, Ford-Hutchinson AW. Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. FEBS Lett 1993; 330: 156-60
31. Davis R, Brodgen RN. Nimesulide. An update of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs 1994; 48: 431-54
32. Tassorelli C, Joseph SA. Systemic nitroglycerin induces Fos immunoreactivity in brainstem and forebrain structures of the rat. Brain Res 1995; 682: 167-78
33. Tassorelli C, Joseph SA, Buzzi G, et al. The effect on the central nervous system of nitroglycerin – putative

mechanisms and mediators. Prog Neurobiol 1999; 57: 606-24
34. Tassorelli C, Joseph SA, Nappi G. Neurochemical mechanisms of nitroglycerin-induced neuronal activation. Neuropharmacology 1997; 10: 1417-24
35. Tassorelli C, Greco R, Morocutti A, et al. Nitric oxide-induced neuronal activation in the central nervous system as an animal model for migraine: mechanisms and mediators. Funct Neurol 2001; 16 Suppl 4: 69-76
36. Jones MG, Lever I, Bingham S, et al. Nitric oxide potentiates response of trigeminal neurones to dural or facial stimulation in the rat. Cephalalgia 2001; 21: 643-55
37. Reuter U, Chiarugi A, Bolay H, et al. Nuclear factor-kappaB as a molecular target for migraine therapy. Ann Neurol 2002; 51: 507-16
38. Tassorelli C, Greco R, Wang D, et al. Nitroglycerin induces hyperalgesia in rats – a time-course study. Eur J Pharmacol 2003; 464: 159-62
39. D'Armour FE, Smith DC. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1980; 72: 74-9
40. Tjolsen A, Berge OG, Hunskaar S, et al. The formalin test: an evaluation of the method. Pain 1992; 51: 5-17
41. Hao S, Takahata O, Iwasaki H. Intrathecal endorphin-1 produces antinociceptive activities modulated by alpha 2-adrenoceptors in the rat tail flick, tail pressure and formalin tests. Life Sci 2000; 66: 195-204
42. Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. Nature 1983; 306: 686-8
43. Sengupta S, Velpandian T, Sapra P, et al. Comparative analgesic efficacy of nimesulide and diclofenac gels after topical application on the skin. Skin Pharmacol Appl Skin Physiol 1998; 11: 273-8
44. Gupta SK, Bansal P, Bhardwaj RK, et al. Comparative antinociceptive, anti-inflammatory and toxicity profile of nimesulide vs nimesulide and piperine combination. Pharmacol Res 2000; 41: 657-62
45. Matsumoto H, Naraba H, Ueno A, et al. Inhibition of cyclooxygenase-2 causes an enhancement of writhing response in mice. Eur J Pharmacol 1998; 352: 47-52
46. Bianchi M, Broggini M. Anti-hyperalgesic effects of nimesulide: studies in rats and humans. Int J Clin Pract 2002; 128 Suppl. 1: 11-9
47. Islas-Cadena M, Banuelos P, Granados-Soto V. Evidence for the participation of the nitric oxide-cyclic GMP pathway in the antinociceptive effect of nimesulide. J Pharmacol Toxicol Metab 1999; 42: 87-92
48. Torfgard K, Ahnler J, Axelsson KL, et al. Tissue distribution of glyceryl trinitrate and the effect on cGMP levels in rat. Pharmacol Toxicol 1989; 64: 369-72
49. Mashimo T, Pak M, Choe H, et al. Effects of vasodilators guanethidine, nicardipine, nitroglycerin, and prostaglandin E1 on primary afferent nociceptors in humans. J Clin Pharmacol 1997; 37: 330-5
50. Sandrini G, Tassorelli C, Proietti Cecchini A, et al. Effects of nimesulide on nitric oxide-induced hyperalgesia in humans – a neurophysiological study. Eur J Pharmacol 2002; 450: 259-62
51. Taniguchi Y, Yokoyama K, Inui K, et al. Inhibition of brain cyclooxygenase-2 activity and the antipyretic action of nimesulide. Eur J Pharmacol 1997; 330: 221-9
52. Taniguchi Y, Ikesue A, Yokoyama K, et al. Selective inhibition by nimesulide, a novel non-steroidal anti-inflammatory drug, of prostaglandin endoperoxidase synthase-2 activity in vitro. Pharmacol Sci 1995; 1: 173-5
53. Vago T, Bevilacqua M, Norbiato G. Effect of nimesulide action time dependence on selectivity towards prostaglandin G/H synthase/cyclooxygenase activity. Arzneimittelforschung 1995; 45: 1096-8
54. Beiche F, Geisslinger G, Goppelt-Struebe M. Expression of cyclooxygenase isoforms in the rat spinal cord and their regulation during adjuvant-induced arthritis. Inflamm Res 1988; 47: 482-7
55. Maihofner C, Tegeder I, Euchenhofer C, et al. Localization and regulation of cyclo-oxygenase-1 and -2 and neuronal nitric oxide synthase in mouse spinal cord. Neuroscience 2000; 101: 1093-108
56. Tegeder I, Niederberger E, Vetter G, et al. Effects of selective COX-1 and -2 inhibition on formalin-evoked nociceptive behavior and prostaglandin E_2 release in the spinal cord. J Neurochem 2001; 79: 777-86
57. Torres-Lopez JE, Ortiz M, Castaneda-Hernandez G, et al. Comparison of the antinociceptive effect of celecoxib, diclofenac and resveratrol in the formalin test. Life Sci 2002; 70: 1669-76
58. Euchenhofer C, Maihofner C, Brune K, et al. Differential effect of selective cyclooxygenase-2 (COX-2) inhibitor NS 398 and diclofenac on formalin-induced nociception in the rat. Neurosci Lett 1998; 248: 25-8
59. Mazario J, Gaitan G, Herrero JF. Cyclooxygenase-1 vs. cyclooxygenase-2 inhibitors in the induction of antinociception in rodent withdrawal reflexes. Neurpharmacology 2001; 40: 937-46
60. Yaksh TL, Dirig DM, Conway CM, et al. The acute antihyperalgesic action of nonsteroidal, anti-inflammatory drugs and release of spinal prostaglandin E_2 is mediated by the inhibition of constitutive spinal cyclooxygenase-2 (COX-2) but not COX-1. J Neurosci 2001; 21: 5847-53
61. Yamamoto T, Nozaki-Taguchi N. The role of cyclooxygenase-1 and -2 in the rat formalin test. Anesth Analg 2002; 94: 962-7
62. Coderre TJ, Katz J, Vaccarino AL, et al. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. Pain 1993; 52: 259-85
63. Sasaki M, Tohda C, Kuraishi Y. Region-specific increase in glutamate release from dorsal horn of rats with adjuvant inflammation. Neuroreport 1998l; 9: 3219-22
64. Sluka KA, Willis WD. Increased spinal release of excitatory amino acids following intradermal injection of capsaicin is reduced by a protein kinase G inhibitor. Brain Res 1998; 798: 281-6
65. DeGroot J, Zhow S, Carlton SM. Peripheral glutamate release in the hind paw following low and high intensity sciatic stimulation. Neuroreport 2000; 14: 497-502
66. Lawand NB, McNearney T, Westlund KN. Amino acid release into the knee joint: key role in nociception and inflammation. Pain 2000; 86: 69-74
67. Ferreira SH, Lorenzetti BB. Intrathecal administration of prostaglandin E_2 causes sensitization of the primary afferent neuron via the spinal release of glutamate. Inflamm Res 1996; 45: 499-502

68. Kawamata T, Omote K. Activation of spinal N-methyl-D-aspartate receptor stimulates a nitric oxide/cyclic guanosine 3,5-monophosphate/glutamate release cascade in nociceptive signaling. Anesthesiology 1999; 91: 1415-24
69. Dickenson AH. A cure for wind-up: NMDA receptor antagonists as potential analgesics. Trends Pharmacol Sci 1990; 11: 307-9
70. Minami T, Nishihara I, Uda R, et al. Involvement of glutamate receptors in allodynia induced by prostaglandin E_2 and F_2 alpha injected into conscious mice. Pain 1994; 57: 225-31

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