Biphasic Response to Nitric Oxide of Spinal Trigeminal Neurons With Meningeal Input in Rat—Possible Implications for the Pathophysiology of Headaches

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NITRIC OXIDE (NO) is involved in the pathogenesis of chronic headaches. Infusion of NO donors can trigger headache attacks, and products of NO metabolism are found to be increased in the cranial circulation in patients suffering from such headaches. To examine if NO is involved in mediating and maintaining spinal trigeminal neuronal activity, an animal model of meningeal nociception was used. In barbiturate-anesthetized rats, a cranial window was made to expose the parietal dura mater. An access to the medullary brain stem allowed extracellular action potentials to be recorded from neurons in the spinal trigeminal nucleus that received afferent input from the exposed dura. Slow intravenous infusion of the NO donor, sodium nitroprusside (SNP, 50 μg/kg), transiently increased spontaneous activity in a subset of neurons and, with a latency of 50 min, caused a progressive increase in impulse activity across the entire sample of neurons. A similar pattern of delayed activation was seen after topical application of the same dose of SNP onto the exposed medulla. Slow injection of the nonspecific inhibitor of NO synthase, Nω-nitro-l-arginine methyl ester (20 mg/kg), reduced the spontaneous activity in all neurons within 15 min. The results suggest that NO can induce delayed, slowly developing activation of central trigeminal neurons and that endogenous release of NO may contribute to the ongoing activity of these neurons. The delayed changes in neuronal activity may include gene expression of pro-nociceptive mediators. These mechanisms may be relevant for the pathogenesis of chronic headaches.

INTRODUCTION

Clinical observations and experimental data suggest that nitric oxide (NO) is involved in the pathogenesis of chronic headaches. A well-known unpleasant side effect of the NO donor glyceryl trinitrate (nitroglycerin, GTN), used therapeutically as a coronary vasodilator, is headache (Dalsgaard-Nielsen 1955). Intravenous infusion of GTN can immediately induce headache both in healthy subjects and patients suffering from primary headaches (Dalsgaard-Nielsen 1955; Iversen et al. 1989; Olesen et al. 1993). This early headache has been described to be of mild to moderate intensity in healthy persons, whereas patients suffering from migraine and tension-type headaches respond with significantly higher pain scores (Olesen et al. 1993). Moreover, in migraine patients, this early headache is followed by a second headache phase that occurs several hours after the infusion of GTN and frequently fulfills the criteria of a genuine migraine attack (Thomsen et al. 1994). Similarly, in patients suffering from cluster headache, headaches resembling cluster attacks have been provoked within 1 h of sublingual administration of GTN (Ekborn 1968). Olesen and colleagues proposed therefore that NO derived from GTN is not only a key molecule in these experimental headaches but, as an endogenous signal substance, may play a causative role in migraine and other vascular headaches (Olesen et al. 1994). It was further proposed that inhibitors of NO synthesis or NO scavengers may be effective in the acute treatment of migraine because the infusion of a NO synthase inhibitor was able to relieve ongoing migraine attacks (Lassen et al. 1997, 1998) and to reduce the severity of chronic tension-type headaches (Ashina et al. 1999). Although NO concentrations cannot be directly measured due to the extremely short half-life of this molecule, there is clear evidence for an increase in NO production in different types of chronic headaches. Increased concentrations of nitrates and nitrates, products of the NO metabolism, have been found in blood samples taken from the internal jugular vein of migraineurs during their attacks (Sarchielli et al. 2000; Shimomura et al. 1999). Recent studies have also shown increased concentrations of nitrates in blood plasma of migraineurs with and without aura as well as cluster headache patients even between attacks (D’Amico et al. 2002). Similarly, the cerebrospinal fluid of patients suffering from chronic daily headache has recently been found to contain elevated concentrations of nitrates (Gallai et al. 2003). Thus it seems that increased NO concentrations are typical if not causative for the development of primary headaches.

The mechanisms underlying the induction of delayed headache after GTN infusion or endogenous increases in NO turnover are not yet sufficiently determined. Early experimental studies in animals indicated that gene expression in the trigeminal system may be involved. Systemic infusion of large doses of GTN have been shown to provoke c-fos expression, a marker for predominantly nociceptive input, in the caudal trigeminal nucleus of the rat (Pardutz et al. 2000; Tassorelli and Joseph 1995a,b). This response was reduced by pretreatment with the nonspecific NO synthase inhibitor Nω-nitro-l-arginine methyl ester (L-NAME) (Tassorelli et al. 1997). Using an electrophysiological approach in the rat, infusion of GTN...
has been shown to reduce the activation threshold of trigeminal neurons and to potentiate responses to electrical stimulation of facial areas and the cranial dura mater (Jones et al. 2001). In the same study, GTN alone did not change c-fos expression but was able to augment c-fos expression provoked by intradermal injection of capsaicin in the periorbital area (Jones et al. 2001). Conversely, in a recent study in the rat, we have shown that infusion of the NO synthase inhibitor L-NAME is able to reduce the spontaneous activity of neurons in the caudal trigeminal nucleus that receive sensory input from the cranial dura mater (De Col et al. 2003).

In the present work, we examined the effects of both intravenous and local (intrathecal) application of the NO donor sodium nitroprusside (SNP) on the spontaneous activity of neurons in the caudal trigeminal nucleus with afferent input from the cranial dura mater. To simulate ongoing nociceptive input from the meninges, repetitive electrical stimulation of the exposed cranial dura was performed in an additional group of experiments. In a third group, we extended our previous study looking at the effect of infusion of L-NAME.

**METHODS**

*Anesthesia and general animal treatment*

The experiments were performed in accordance with the ethical guidelines of the International Association for the Study of Pain (Zimmermann 1983). The experimental protocol was reviewed by an ethics committee and approved by the local government. Male Wistar rats with body weights ranging from 270 to 440 g were used. Details of the surgical and recording procedures have been described elsewhere (Scheipelmann et al. 1999). Briefly, rats were anesthetized with an initial intraperitoneal dose of 120–150 mg/kg thiopental. The femoral artery was cannulated to monitor mean arterial blood pressure, and the femoral vein was cannulated for the administration of drugs. The animals were tracheotomized, paralyzed with intravenous administration of gallamine triethiodide (40 mg/kg), and artificially ventilated with oxygen-enriched room air. Expiratory CO₂ was monitored and maintained at 4.5–5.5%. Body temperature was maintained at 37–37.5°C with a feedback-controlled homeothermic system (TKM 0902, FMI GmbH). The animals were kept under deep anesthesia throughout the experiment with supplemental doses of thiopental given as required to suppress nociceptive reflexes or blood pressure changes evoked by noxious pinch stimuli of the hindpaw and the earlobe. Vital parameters (blood pressure, heart rate, expiratory CO₂ level, and body temperature) were continuously recorded throughout the experiment. To end an experiment, the rat was killed either with a lethal dose of intravenous thiopental or by perfusion of fixation after an additional dose of thiopental.

*Head surgery and electrophysiological recordings*

The animal was placed in a stereotaxic frame with the head held in a fixed position by ear bars. The skin overlying the skull was opened and the cranium exposed. Using a drill and fine forceps, a cranial window ~6 mm (rostrocaudal) and 4 mm (inferior-superior) was carefully cut into the left parietal bone to expose the dura mater. During surgery and throughout the experiment, the dura was protected from drying with isotonic saline. The neck muscles were divided along the animal’s midline, and the medullary brain stem was exposed by cutting a window into the atlanto-occipital ligament and the underlying dura mater. Carbon fiber glass microelectrodes (impedance: 1–6 MΩ) were inserted into the ipsilateral medulla and advanced or retracted in 2.5-μm steps using a microstepper. Single shock stimuli (0.5-ms pulses, 10–12 V, 0.2 Hz) were delivered to the exposed dura through bipolar electrodes with their rounded tips touching the dural surface. Neurons in the subnucleus caudalis of the spinal trigeminal nucleus (STN) with input from meningeal afferents were identified by their regular discharge in response to electrical stimulation of the dura with a minimal latency that was fairly stable (but varied ~4 ms in neurons with high spontaneous activity) and by their responses to mechanical probing of the dura. The position and size of dural receptive fields were determined with von Frey filaments (5–10 mN), and facial receptive fields indicating convergent afferent input were located with a fine blunt glass rod (Fig. 2/2 insert). The extracellular signals were band-passed, amplified, and digitized (sampling rate: 20 kHz) before being recorded to disk using SPIKE software (Forster and Handwerker 1990). The position of each recording site was determined by measuring the distance caudal and lateral to the obex of the electrode and by reading the depth of the microdrive. In some experiments, the recording site was marked by passing positive current through the microelectrode (50 μA for 30 s) to produce a detectable lesion (Fig. 1B). The animals in which such lesions were made were thoracotomized and perfused, through a catheter inserted into the left ventricle, with saline followed by 4% formaldehyde. Cryostat sections (20 μm) were cut from the medulla and Nissl stained to identify the lesion.

**Experimental procedures**

Throughout the experiment, the number of impulses was counted at intervals of 1 s. The spontaneous activity of all units was recorded for a control period of ~30 min before application of any substance. After the control period, SNP (50 μg/kg, Sigma) dissolved in 1 ml saline was intravenously infused over a period of 10 min. This dose was calculated to cause a blood plasma concentration of ~5 μM (assuming a plasma volume of 3% of the body weight). In previous experiments, local concentrations of 1–10 μM SNP applied onto the cranial dura caused small increases in meningeal blood flow indicating a mild vascular effect, whereas higher concentrations caused significant and long-lasting effects. (Messlinger et al. 2000).

Using the same protocol, control experiments were made with SNP that had been photodegraded for 2 days at room temperature in a test tube (Ikeda et al. 1988). In five other control experiments, the effect of a systemic blood pressure decrease on the neuronal activity was examined. The nonspecific α-receptor antagonist phentolamine (1 mg/kg iv) was injected over a few minutes at a rate that initially depressed the systemic pressure by ~20 mmHg.

In another set of experiments, the cerebrospinal fluid covering the medullary brain stem was replaced by 50 μg/kg SNP, dissolved in 0.2 ml saline, and slowly applied onto the medulla over a period of ~3 min.

In eight experiments, the dura was repetitively stimulated with electrical pulses (0.5 ms, 5 Hz) at a stimulation strength of two to three times threshold (8–13 V). Beginning one minute after the intravenous administration of SNP, periods of 1-min stimulation were applied at 10-min intervals for the subsequent 2 h.

In a different set of experiments, after a control period of 30 min, a 0.5 ml saline solution containing L-NAME 20 mg/kg (Sigma) was slowly intravenously injected over a period of 60 s. In these experiments, neuronal activity was recorded for a period of 20 min before to 90 min after the L-NAME injection.

**Data analysis**

Action potential waveforms were analyzed off-line for each neuron using the SPIIDI software package (Forster and Handwerker 1990). Spike frequency was normalized to the average rate of activity during the last 10 min of the control period before administration of substances. Averaged activity during each subsequent 10-min interval was statistically compared with the control interval before SNP.
administration using a t-test for dependent samples. In datasets containing the full observation period in all neurons, ANOVA with repeated measurements, extended by Fisher’s least-square different (LSD) test, was used instead of the t-test. To evaluate changes in activity after application of substances in single neurons, 1-min intervals of activity were compared. If the discharge frequency exceeded the mean activity of the control period by >2 SDs, it was considered a short-term effect. Pairwise correlations between location coordinates, latency to electrical stimulation, spontaneous activity and relative changes of activity after treatment with SNP and L-NAME, were calculated. Data are given in mean ± SE if not designated otherwise. All statistical tests were performed using the STATISTICA 6.0 (StatSoft, Tulsa, OK) software package.

**RESULTS**

**General properties of neurons**

A total of 54 units recorded in the left caudal subnucleus of the STN were included in this study. The recording sites were located 0.90/2.16/3.22 mm (min/mean/max) caudal to the obex and 0.50/1.40/2.50 mm lateral from the midline and at a depth of 0.06/0.95/1.55 mm from the dorsal surface of the medulla (Fig. 1A). The measured recording position was confirmed in four experiments by an electrolytic lesion visible in Nissl-stained serial sections (Fig. 1B). The electrical threshold, at which discharges could be evoked by electrical stimulation of the dura mater, was between 1.5 and 6 V. At threshold, the minimal response latency to dural electrical stimulation varied from 6 to 22 ms, indicating afferent input from C and Aδ fibers. In most units, only one response was elicited by electrical stimulation, even at stimulation intensities of two to three times the threshold intensity. There was a clear correlation between latencies and location of neurons (r = −0.57; P < 0.001). Neurons located more caudal to the obex had a shorter stimulus-response latency. Dural receptive fields were usually spot-like and located close to branches of the middle meningeal artery. Dural von Frey thresholds varied between different units from 4.5 to 56 mN. Most had low mechanical threshold facial receptive fields predominantly in the ophthalmic region as has been found in previous studies (Schepelmann et al. 1999; Yamamura et al. 1999). Two units could only be activated by strong pressure within a small facial area. The rate of mean spontaneous activity between units varied from less than 1 imp/min (minimum) to 3,270 imp/min (maximum), and the mean rate was 243.6 ± 94.4 imp/min. During the control period or within a longer observation time with topical or intravenous injection of saline the activity of units was fluctuating without preference.

**Response to intravenous administration of SNP**

After a control period of 30 min, intravenous injection of SNP (50 μg/kg) over 10 min produced either an increase or no change (Fig. 2A) in the ongoing activity of 14 neurons. For the whole sample, this increase in activity was significant when the activity was calculated in 10-min periods (repeated-measures ANOVA; Fig. 3A). After a latency of ~50 min after SNP administration, the mean rate of spontaneous activity increased significantly (P = 0.05, repeated-measures ANOVA; Fig. 3A), and after 70 min this increase was nearly two times the rate of control activity (Figs. 2A and 3A). Eight single neurons were observed over a period of >2 h. Five of these units showed significant increases in spontaneous activity after SNP infusion (P < 0.05, t-test, 30 min before vs. 120 min after SNP) while no increase was seen in the other units. The relative change in activity was not correlated with the initial spontaneous activity nor was there any correlation of the response to SNP with other physiological properties of the units.

To test if sodium ferricyanide, another product of SNP breakdown, may change the activity of neurons, a separate series of four control experiments was performed with SNP that had been photodegraded. Infusion of photodegraded SNP (50 μg/kg) was not followed by significant long-term changes in either neuronal activity or blood pressure (Fig. 4A). In two
of these experiments, injection of the substance was accompanied by a short increase in spontaneous activity, but for the whole sample this effect was not significant. To test if the decrease in blood pressure caused by SNP may change the activity of neurons, the nonspecific α-receptor antagonist phentolamine (1 mg/kg iv) was slowly injected in another series of five control experiments. Phentolamine caused an initial steep decrease in blood pressure that did not fully recover within two hours (Fig. 4B). Neither the large initial fall in pressure nor the

**FIG. 2.** Example recordings of spontaneous activity from neurons in the spinal trigeminal nucleus with spike forms (left insets), facial receptive fields (middle inset), and meningeal receptive fields (right insets). A: experiment with intravenous infusion of 50 μg/kg sodium nitroprusside (SNP). B: experiment with topical application of SNP onto the medulla (i.t.). C: control experiment with intravenous infusion of photodegraded SNP.

**FIG. 3.** Normalized mean ± SE activity of neurons treated with 50 μg/kg SNP. A: sample of neurons treated with intravenous infusion of SNP. B: sample of neurons treated with intravenous injection of SNP and repetitive electrical stimulation (flashes). C: sample of neurons treated with topical administration (i.t.) of SNP onto the medulla. Significant increases in activity (*) compared with the activity before SNP.
Persisting decrease by \(-5\) mmHg on average caused significant changes in neuronal activity (Fig. 4B).

### Combination of intravenous SNP and dural electrical stimulation

In a separate sample of eight units, SNP infusion was combined with electrical stimulation applied at 5 Hz for periods of 1 min. The electrical stimuli were added to test whether the effect of delayed activation after SNP is influenced by repetitive peripheral stimulation mimicking an ongoing nociceptive input. An increase in spontaneous activity was registered during a period of 80–110 min after SNP infusion (\(P < 0.05\), t-test with dependent samples; Fig. 3B). The time course of this response did not significantly differ from the pattern observed with SNP infusion alone (2-way ANOVA with repeated measures, comparing subsequent 10-min intervals in both samples).

### Response to intrathecal administration of SNP

In a further 11 units, SNP was topically applied onto the medulla (intrathecally). During or within a period of 5 min after SNP application a short-term increase in activity was observed in five units (Fig. 2B). After a latency of 60 min after SNP administration, the mean rate of spontaneous activity increased significantly (10-min intervals, repeated-measure ANOVA), lasting until 100 min after the SNP administration (Fig. 3C). Separate analysis in single neurons revealed that this effect was due to six units with a significant increase in spontaneous activity (\(P < 0.05\), t-test, 1-min intervals within the 30-min interval before vs. 90- to 120-min interval after SNP).

### Response to administration of L-NAME

In a separate sample of 12 units, L-NAME (20 mg/kg iv) was injected over a period of 60 s. This treatment caused an elevation of the mean arterial blood pressure within five minutes from 107 ± 0.6 to 127 ± 5.0 mmHg (\(P < 0.001\), \(n = 5\), t-test using 10 1-min intervals; Fig. 5). An initial increase in...
activity, i.e., exceeding the mean plus two SDs, in the 2-min period immediately after L-NAME administration was seen in 8 of 12 units. This short-term effect was not significant when all neurons were averaged. Four minutes after L-NAME injection, the spontaneous activity decreased ($n = 12$, $P < 0.05$ for all minute periods, repeated-measure ANOVA; Fig. 5). This decrease in activity persisted in 12 units for the 17-min observation period after L-NAME injection (Fig. 5). In three units that were observed for 2 h after L-NAME administration, the decreased activity further persisted within this period. In five neurons of this sample, SNP was intravenously applied at different time points after L-NAME. This SNP injection caused a brief activation but did not reverse the (protracted) decrease in spontaneous activity ($n = 5$, data not statistically analyzed due to varying time points).

**DISCUSSION**

The main observation in this study was an increase in the spontaneous activity of trigeminal neurons with meningeal afferent input induced by the NO donor SNP. The latency of this effect was $\sim 1$ h, both after intravenous and local application of SNP onto the medullary brain stem. The latency of the SNP induced increase in activity was not significantly altered with the addition of electrical stimulation of the dura. This observation suggests that either or both peripheral and central mechanisms are induced by NO that in turn produces delayed and long-lasting pro-nociceptive changes in neurons in the trigeminal nucleus. The majority but not all neurons showed delayed activation after SNP, and there was no correlation between the response to SNP and other physiological properties of the units. The reason for this differential SNP effect is not clear. The second important observation in this study is that the unspecific inhibitor of NO synthases, L-NAME, reduced the spontaneous activity in these neurons within some minutes, suggesting that the spontaneous activity is partly dependent on continuous NO production. An additional observation was that electrical stimulation of the dura mater simulating noxious input was not able to further increase or to prevent this increase in neuronal activity caused by infusion of the NO donor.

**General properties of neurons in the spinal trigeminal nucleus**

All neurons included in this study were located in or near the caudal subnucleus of the spinal trigeminal nucleus. All neurons had convergent input from the parietal dura and from facial areas. Other properties such as recording depth, response latency to electrical stimuli, spontaneous activity, and activation threshold for dural mechanical stimulation were highly variable between the units as was the response to infusion of the NO donor. Therefore we looked for correlations between these general properties. The only correlation found was between the coordinates of the units’ position and their response latency to electrical stimulation of the dura mater. There was a significant negative correlation of the latencies with the rostrocaudal coordinates of the units. This observation may indicate that intracranial trigeminal afferents project differentially to the STN, e.g., the most caudal part of the STN may receive more A6 fiber input, or more primary afferents may directly reach the caudal STN compared with the rostral parts.

There was also no correlation of these properties with the changes in neuronal activity after SNP infusion. In particular, the changes in activity induced by the NO donor and by the NOS inhibitor were not dependent on the initial activity of the neurons. This is important because initial high spontaneous activity could have been due to sensitization processes during the surgical procedures, which may also include NO-dependent mechanisms.

**Downregulation of neuronal activity by L-NAME**

In our previous study, L-NAME infusion decreased spontaneous activity in a sample of neurons in deep laminae of the caudal trigeminal nucleus, suggesting that NO is involved in the control of trigeminal neuronal activity (De Col et al. 2003). In the present study, we extended these experiments by including additional neurons located in more superficial laminae of the spinal trigeminal nucleus. The results of this study are in full accordance with our previous findings. There was no systematic variation in the response to L-NAME depending on the location of neurons. Although the preparation used in this study is not a complete model for migraine headache, our findings resemble clinical studies in which the severity of pain during spontaneous migraine attacks was significantly reduced by inhibition of NO synthase (Lassen et al. 1997, 1998; Olesen and Jansen-Olesen 2000; van der Kuy et al. 2002).

It is unclear so far which of the three isoforms of NO synthase, eNOS, iNOS, or nNOS (Moncada et al. 1991), is mainly responsible for the endogenous production of NO that obviously contributes to the maintenance of neuronal activity in the experimental situation. L-NAME is a nonspecific NOS inhibitor that blocks all three types of NOS and reduces NO production in many tissues including vascular endothelium leading to arterial vasoconstriction. The onset of blood pressure increase was associated with a transient increase in neuronal activity. Although the systemic pressure continued to be elevated after injection of L-NAME, the neuronal activity decreased and remained suppressed (see Fig. 5). Injections of SNP under this condition caused short-lasting increases in activity, similar to that observed in some experiments with initial SNP infusion, but the decrease in spontaneous activity caused by L-NAME was not reversed, indicating that NO derived from the applied SNP was not able to compensate for the blockade of endogenous NO production.

**Acute effect of intravenous and intrathecal administration of SNP**

Under the experimental conditions of slow intravenous SNP infusion, activation was observed in 7 of 14 neurons. Similarly, during or within the 5-min period after intrathecal SNP application, activation was seen in 5 of 11 units; however, across the whole sample, this effect failed to reach the level of significance. Fast nociceptive effects of NO donors in the trigeminal system have also been reported by other authors. Rat trigeminal nucleus neurons were sensitized to electrical stimulation of facial and dural areas during or immediately after the infusion of nitroglycerin, a NO donor that, unlike SNP, does not produce cyanide (Jones et al. 2001). In experiments on humans, intravenous nitroglycerin immediately provoked some form of mild headache in 9 of 10 healthy subjects (Iversen et
al., 1989). The mechanism underlying this rapid onset of the NO effect is unclear. Mechanical activation of meningeal afferents by rapid vasodilatation is possible although not very likely because injection of phenolamine, which caused vasodilatation and a short-term decrease in blood pressure, was not accompanied by any significant changes in neuronal activity. Another possible mechanism is a direct effect of NO on trigeminal neurons or meningeal afferents. In this case, either the NO concentration produced by SNP infusion was too low to directly activate all neurons or not all neurons were sensitive to NO.

Delayed effect of intravenous and intrathecal administration of SNP

Application of the NO donor SNP was followed by an increase in spontaneous neuronal activity in most neurons with a delay of ~1 h. These results are in good accordance with the clinical “migraine model” in which an infusion of the NO donor nitroglycerin provoked migraine attacks with a similar delay of one to several hours (Bank, 2001; Iversen et al., 1989; Olesen et al., 1993; Peters, 1953). Due to the short half-life of NO donors in the blood plasma, this effect cannot be explained by a direct activation of trigeminal neurons; rather the NO derived from SNP or nitroglycerin, respectively, is presumed to have triggered a long-term process. We can only speculate about the mechanisms underlying this process. NO, a vasoactive substance and a neuronal messenger (Garthwaite and Boulton, 1995; Tassorelli et al., 1999), may act both on cerebral blood vessels leading to vasodilatation and on perivascular sensory endings to release neuropeptides and prostaglandins (Holzer, 1995; Strecker, et al., 2002). As was hypothesized by some authors, COX and NOS pathways may interact and facilitate each other, and this mutual facilitation could contribute to the pain production in migraine (Sarchielli et al., 2000; Stirparo, et al., 2000). It is known that most of the NO effects are mediated by activation of soluble guanylate-cyclase, which stimulates synthesis of cyclic guanosine monophosphate (Mayer, 1994; Riedel, 2000). NO is also able to stimulate neurotransmitter release (see reviews by Meller and Gebhart, 1993; Wang and Robinion, 1997) and to regulate neurotransmitter (glutamate, dopamine) reuptake (Pogun and Kuhar, 1993; Holzer, 1995); Riedel, 1997). The contribution of these mechanisms to the effects observed in our experiments cannot be excluded, but none of these mechanisms work over a time course longer than a few minutes. Therefore other long-term effects must be assumed to underlie the delayed activation of trigeminal neurons, and therefore the most likely possibility is the involvement of gene expression.

It has been reported that treatment of cultured bovine pulmonary artery endothelial cells with NO-donors (S-nitroso-N-acetylpenicillamine, sodium nitroprusside, or spermine NONOate) stimulates endothelial NO synthase (eNOS) expression (Chen, et al., 2001). Systemic injection of the NO-donor nitroglycerin has been shown to increase neuronal NO synthase (nNOS) levels in the caudal trigeminal nucleus in rats (Pardutz, et al., 2000). Moreover, expression of inducible NO synthase (iNOS) has recently been found in the rat dura mater 4 h after intravenous GTN infusion, although this was due to the invasion of macrophages (Reuter, et al., 2001). Thus it seems possible that gene expression of all three isoforms of NOS can be induced by NO. If NOS expression occurred in our experiments, subsequent endogenous NO production may have been the mechanism that led to the delayed increase in firing rate of caudal trigeminal nucleus neurons. Assuming that endogenous NO production is a source of delayed neuronal activation, the question is whether this may occur in the meninges or in the caudal trigeminal nucleus or both.

Peripheral versus central effects of the NO donor

To figure out whether the delayed activation of STN neurons is caused by a peripheral or central effect, we used two modes of SNP application—systemic (intravenous) and direct application onto the medulla (intrathecal). Although NO, generated by intravenous SNP injection, may interact with both central and peripheral neurons, intrathecal application should mainly affect brain stem structures. Because both modes of application produced the same result, we propose that the delayed changes in spinal trigeminal neuronal activity observed after SNP are mainly due to a central action. On the other hand, considering that the increase in neuronal activity was somewhat delayed and that the increase in activity was less pronounced after intrathecal compared with intravenous infusion of SNP, a contribution of peripheral effects of NO cannot be dismissed. In an in vitro preparation of the rat meninges, NO donors caused the release of calcitonin gene-related peptide and prostaglandin E2 (Strecker, et al., 2002), supporting the idea of an additional peripheral effect of NO on meningeal primary afferents.

To further test the possibility that the increase in firing rate of spinal trigeminal neurons after SNP administration is a result of enhanced peripheral input from meningeal afferent fibers, we combined intravenous SNP administration with repetitive electrical stimulation of the dura mater. The effect of this combination was not significantly different from the experiments with intravenous SNP injection alone. This led us to conclude that regular stimuli that may activate trigeminal afferents, such as arterial pulsation, seem not to be involved in the delayed activation of trigeminal nucleus neurons by SNP. On the other hand, we cannot exclude the possibility that it was the 5-Hz stimulation frequency that caused depression rather than potentiation of activity in second-order neurons. In any case, we assume that it is mainly a central action by which systemic NO donors change the activity of trigeminal second-order neurons.

In conclusion, we suggest that systemic or central administration of NO donors may trigger a process that leads to a delayed and long-lasting activation of spinal trigeminal neurons resulting in an increase in ongoing impulse activity. This process is most likely central in origin and may include expression of NOS, possibly nNOS, in the STN. We speculate that similar mechanisms underlie the induction of experimental migraine and that these may play a role in the onset and maintenance of spontaneous migraine headache as has been previously proposed (Olesen et al., 1993–1995, Olesen and Jansen-Olesen, 2000).

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