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The effect of anti-migraine compounds on nitric oxide-induced dilation of dural meningeal vessels ☆

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Abstract

Migraine is characteristically accompanied by a throbbing quality of head pain thought to involve trigeminovascular afferents. Administration of nitric oxide (NO) donors provides the most reliable model of migraine induction in humans. The present studies used intravital microscopy to monitor the effect of local meningeal nerve stimulation and NO on dural blood vessels and any modulation of that effect by anti-migraine compounds. NO caused an immediate and reproducible dilation of meningeal blood vessels that was partially blocked by sumatriptan and indomethacin, while flunarizine and histamine H_1 and H_2 receptor antagonists were unable to block the dilation. Indomethacin also inhibited the neurogenic dilation while flunarizine did not. The present studies demonstrate that NO is unlikely to interact with histamine to produce its dilatory response. Sumatriptan and indomethacin inhibit the NO response by inhibiting trigeminal activation and calcitonin gene-related peptide (CGRP) release. Flunarizine does not modify either the neurogenic vasodilator response or the NO meningeal dilator response at least acutely.

Keywords: Migraine; Nitric oxide (NO); Meningeal artery, middle; Intravital microscopy; Trigeminal

1. Introduction

Migraine is characteristically accompanied by a throbbing quality of head pain whose pathophysiology, although not fully understood, is thought to include activation of trigeminal afferents (Goadsby et al., 2002). The mechanism of action of anti-migraine compounds, or compounds that trigger attacks, is of interest in the context of developing new treatment strategies. Williamson et al. (1997a) developed an intravital microscopy technique that allows continual observation of dural blood vessels so any changes in meningeal vessel diameter during intravenous administration of drugs can be observed. This technique also allows direct observation of changes in meningeal vessel diameter during dural electrical depolarisation of trigeminal fibres known in humans to be pain-producing (Wolff, 1948), providing a means of studying trigeminovascular dural pharmacology in vivo.

Nitric oxide (NO) is a potent endogenous vasodilator with an impressive array of biological actions (Moncada et al., 1991). NO causes headache and migraine in both control volunteers and headache patients, and will also cause a delayed headache that fulfils The International Headache Society criteria for migraine (Headache Classification Committee of The International Headache Society, 1988) in sufferers several hours after the NO infusion (Iversen et al., 1989; Olesen et al., 1993, 1994). NO-induced migraine can be prevented or alleviated by certain established anti-migraine compounds. In nonmigraineurs, NO-induced headache is attenuated by sumatriptan when administered prior to the NO infusion (Iversen and Olesen, 1996). Sumatriptan also inhibits the regional cerebral blood flow increase that was found in the rat to NO donor infusion (Read et al., 1999), although changes in cerebral blood flow itself are not seen in humans with NO donor infusion (White et al., 2000).

Compounds clinically effective against acute migraine, such as 5-$HT_{1B/1D}$ receptor agonists (Williamson et al.,

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1997a,b, 2001a), and μ-opioid receptor agonists (Williamson et al., 2001b) can inhibit neurogenic meningeal dural vasodilatation caused by electrical stimulation. This action is thought to be via inhibition of the release of calcitonin gene-related peptide (CGRP) from trigeminal sensory fibres innervating the cranial blood vessels (Williamson and Hargreaves, 2001). Indomethacin is a nonsteroidal anti-inflammatory drug that is believed to act by inhibiting cyclooxygenase activity that helps produce prostaglandins (Abdel-Halim et al., 1978). It is helpful for some migraineurs and inhibits NO-induced headache (Castellano et al., 1998). However, its mechanism of action in migraine is not completely clear. Flunarizine, a migraine preventative (Leone et al., 1991), whose migraine action may involve inhibition of Ca^{2+} entry across the cell membrane (Geer et al., 1993), may interact with NO generation mechanisms. Flunarizine reduced the relaxation found with electrical stimulation of intramural nerve terminals of arterial strips while relaxation by NO or sodium nitroprusside (NO donor) was not inhibited by flunarizine (Ayajiki et al., 1997).

The purpose of this study was to examine the effects that NO has on dural blood vessel diameter using the intravital microscopy and the effect of anti-migraine compounds given as either a pretreatment or a treatment during the NO response. Histamine infusion can cause headache in humans (Krabbe and Olesen, 1980; Lassen et al., 1995), and the relationship it has with NO is certainly important. Therefore, we applied histamine receptor antagonists to compare and contrast effects in this model with results seen in clinical studies. Finally, we examined the effect of indomethacin and flunarizine on the neurogenic dural vasodilatation since sumatriptan inhibits this response (Williamson et al., 1997a).

2. Materials and methods

2.1. Surgical preparation

Male Sprague–Dawley rats (300–400 g) were anaesthetised throughout the experiments with sodium pentobarbitone (60 mg/kg i.p. and then 18 mg/kg/h i.v. infusion). The left femoral artery and vein were cannulated for blood pressure recording and intravenous infusion of anaesthetic, respectively. Temperature was maintained throughout using a homeothermic blanket system. The rats were placed in a stereotactic frame, the skull exposed and the right parietal bone thinned by drilling with a saline-cooled drill until the blood vessels of the dura were clearly visible through the intact skull.

2.2. Intravital microscopy

The cranial window was covered with mineral oil (37 °C), a branch of the middle meningeal artery viewed using an intravital microscope (Microvision MV2100, UK) and the image displayed on a television monitor. Dural blood vessel diameter was continuously measured using a video dimension analyser (Living Systems Instrumentation, USA), and displayed with blood pressure on a chart recorder and a data analysis system (MI^2, Modular Instruments, UK and CED spike 2 software).

2.3. Experimental protocols

2.3.1. Defining electrical stimulation parameters

In the preparations where electrical stimulation was used to evoke dilation of the dural blood vessels, a bipolar stimulating electrode (NE 200X, Clark Electromedical) was placed on the surface of the cranial window approximately 200 μm from the vessel of interest. The surface of the cranial window was stimulated at 5 Hz, 1 ms for 10 s (Grass Stimulator S88, Grass Instrumentation) with increasing voltage until maximal dilation was observed. Subsequent electrically induced responses in the same animal were then evoked using that voltage (Akerman et al., 2001a). In control experiments, we tested the reproducibility of the neurogenic vasodilator response to four consecutive stimuli in order to determine whether there was any systematic effect over time that might confound the experimental results.

2.3.2. Nitric oxide donor-induced dilation

In the preparations where nitric oxide was used to dilate dural blood vessels, the carotid artery was cannulated. The nitric oxide donor sodium nitroprusside was infused for 15 min via the carotid artery at doses of 0.5 up to 2 μg/kg until the meningeal dilation reached its maximum. Sodium nitroprusside infusion was then repeated after 10 min when the baseline vessel diameter had returned to check the repeatability of using multiple sodium nitroprusside infusions in the same animal.

2.3.3. Effects of anti-migraine compounds on dural vessel diameter after SNP infusion

The effects of sumatriptan, the 5-$HT_{1B/1D}$ receptor agonist (10 mg/kg), on nitric oxide-induced dilation were studied. The compound was administered 10 min after the onset of the nitric oxide infusion. This protocol was also used for flunarizine (5 mg/kg). The effects of indomethacin (3 mg/kg), the cyclooxygenase inhibitor, were studied. The compound was administered 10 min after the completion of the control response to the sodium nitroprusside infusion; this was followed 15 min later by a second infusion. A further 10 min after the completion of this infusion, an increased dose of indomethacin (10 mg/kg) was given, followed by another 15 min of SNP. This protocol was also used for the histamine H_1 receptor antagonist mepyramine (1, 3 and 10 mg/kg) and also the histamine H_2 receptor antagonist famotidine (0.1, 0.3, 1.0 and 3 mg/kg). With mepyramine and famotidine, up to three different doses were administered in one experiment in ascending order.

2.3.4. Effects of anti-migraine compounds on dural vessel diameter after neurogenic stimulation

The effects of indomethacin (3 mg/kg) on neurogenically produced vasodilation were studied. The compound was administered 10 min after the baseline response to electrical stimulation, and a second response elicited after 15 min. A further 10 min after the final electrical stimulation, an increased dose of indomethacin (10 mg/kg) was given, followed by another electrical stimulation after 15 min. This protocol was also used for flunarizine (1, 2.5 and 5 mg/kg). With flunarizine, up to three dosings were administered in one experiment in ascending order.

2.4. Data analysis

The effects of electrical stimulation and nitric oxide donor infusion on dural vessel diameter were calculated as a percentage increase from the pre-stimulation baseline diameter, and the effects of the post-drug stimulation were then compared to the control response elicited after electrical stimulation. Summary data are presented as mean ± S.E.M. Dural vessel diameter was measured in arbitrary units. An analysis of variance (ANOVA) with repeated measures design (SPSS v10.0) was employed to examine the effect of control vasodilations and test compounds. Paired Student's *t*-test was used for post-hoc testing. Significance was assessed at the $P < 0.05$ level.

2.5. Drugs

Sumatriptan was purchased as succinate (Imigran, Glaxo Wellcome). Indomethacin (Sigma-Aldrich, UK) and flunarizine dihydrochloride (RBI, UK) were dissolved in 45% w/v 2-hydroxy-β-cyclodextrin (RBI). Pyrilamine maleate (mepyramine—RBI) was dissolved in 0.9% saline and famotidine (ICN, UK) was dissolved in water.

3. Results

3.1. Effects of a nitric oxide donor and electrical stimulation on dural vessel diameter

Sodium nitroprusside produced an increase in vessel diameter of 104 ± 7% ($n = 29$) for the length of the infusion (15 min) as a total across all experiments (Fig. 1). This was accompanied by a decrease in arterial blood pressure that returned to normal after the infusion. In the control studies with sodium nitroprusside, the mean dilations were 100 ± 8%, 103 ± 5%, 87 ± 3% and 87 ± 10%, respectively ($n = 6$). There was no difference across the cohort (F (3, 15) = 1.756, $P = 0.199$). In the control studies, four consecutive electrical stimuli were carried out in seven animals with similar intervals to those used in the active pharmacological studies. The mean dilations were 135 ± 7%, 135 ± 9%, 130 ± 11% and 136 ± 8%, respectively. There was no difference across the cohort ($F(3,18) = 0.621$, $P = 0.61$). Electrical stimulation had no effect on mean arterial blood pressure. These control dilations were compared with an ANOVA to the control dilations used in the pharmacological studies, and there were no significant differences.

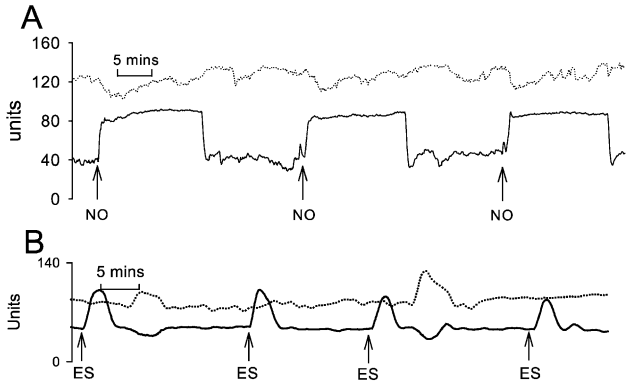

Fig. 1. Original tracing showing the effect of repeated (A) nitric oxide infusions (NO: 0.5–2 μg/kg/min for 15 min) or (B) electrical stimulation (5 Hz, 1 ms for 10 s) on mean arterial blood pressure (dotted line—mm Hg) and vessel diameter (black line—arbitrary units).

3.2. Effect of sumatriptan on dural vessel diameter during SNP infusion

In rats treated with sumatriptan (10 mg/kg), the magnitude of the response to SNP infusion was significantly reduced from 97 ± 14% to 59 ± 18% ($n = 7$, $P < 0.05$). With continued sodium nitroprusside infusion, the magnitude of the vessel dilation increased again to its maximal state (Fig. 2). There was no effect of sumatriptan on mean arterial blood pressure.

3.3. Effect of flunarizine on dural vessel diameter during SNP infusion and neurogenic stimulation

In rats treated with flunarizine (5 mg/kg, $n = 7$), there was no change in the response to SNP infusion, 102 ± 18% to 97 ± 17%. The decrease in vessel diameter returned to its dilated state after 5 min of continued sodium nitroprusside infusion (Fig. 3). Increases in dural blood vessel diameter evoked by electrical stimulation of 68 ± 5% (1 and 2.5 mg/kg, $n = 7$) to 64 ± 5% and 73 ± 10%, respectively, and 90 ± 12% (5 mg/kg, $n = 5$) to 84 ± 5% after 15 min were unaffected by flunarizine (see Fig. 3). Flunarizine had no effect on mean arterial blood pressure.

3.4. Effect of indomethacin on dural vessel diameter during SNP infusion and neurogenic stimulation

In rats pretreated with indomethacin (3 and 10 mg/kg, $n = 6$), the response to SNP infusion was significantly

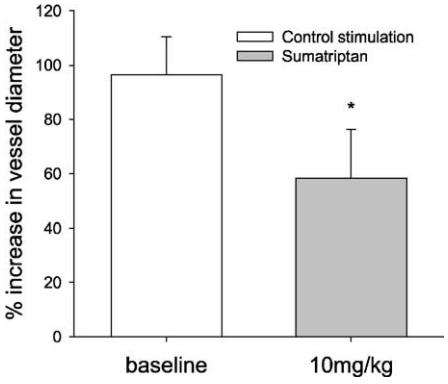

Fig. 2. Effects of sumatriptan (5-$HT_{1B/1D}$ receptor agonist) on nitric oxide-induced dilation. Rats were injected with sumatriptan 10 min into a 15-min nitric oxide (0.5–2 μg/kg/min) infusion. * $P<0.05$.

reduced from 117 ± 20% to 71 ± 10% (3 mg/kg, $P<0.05$) and 55 ± 11% (10 mg/kg, $P<0.05$) (Fig. 4). Increases in dural blood vessel diameter evoked by electrical stimulation were inhibited from 112 ± 9% to 102 ± 12% (3 mg/kg) and 80 ± 13% (10 mg/kg, $P<0.05$). The 10 mg/kg value represents a significant reduction from baseline. Indomethacin caused a slight increase in blood pressure and decrease in baseline that was restored to the baseline value during subsequent infusions.

3.5. Effect of mepyramine on dural vessel diameter with SNP infusion

In rats pretreated with mepyramine (1, 3 and 10 mg/kg, $n=6$), there was no attenuation of the response to sodium nitroprusside infusion, 91 ± 10% to 99 ± 13% (1 mg/kg), 81 ± 14% (3 mg/kg) and 76 ± 6% (10 mg/kg), respectively. Mepyramine had little effect on mean arterial blood pressure.

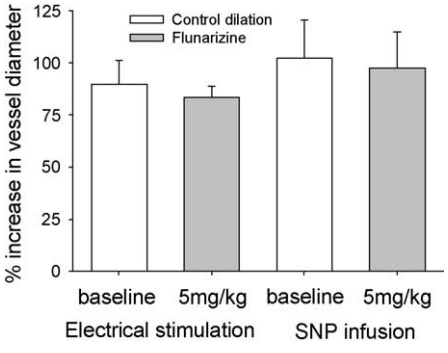

Fig. 3. Effects of flunarizine (Ca^{2+} channel blocker) on nitric oxide-induced dilation and electrical stimulation. Rats were injected with flunarizine 10 min into a 15-min nitric oxide (0.5–2 μg/kg/min) infusion, or following control responses, rats were injected with flunarizine and then electrical stimulation (50–300 μA) was repeated.

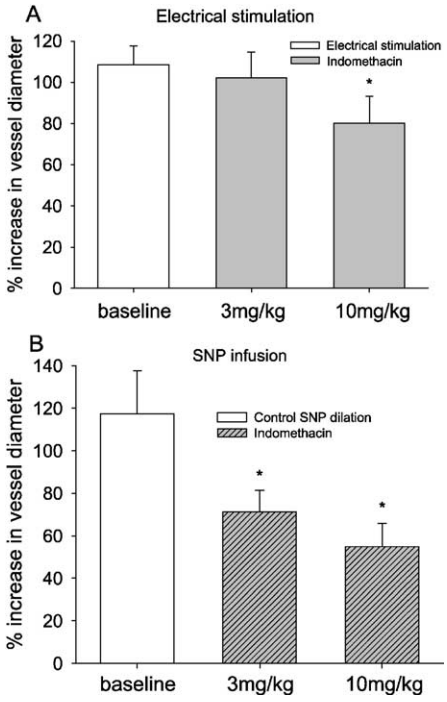

Fig. 4. Effects of indomethacin (cyclooxygenase inhibitor) on (A) electrical stimulation and (B) nitric oxide-induced dilation. Following control responses, rats were injected with indomethacin and then electrical stimulation (50–300 μA) or nitric oxide (0.5–2 μg/kg/min) infusion was repeated. * $P<0.05$.

3.6. Effect of famotidine on dural vessel diameter with SNP infusion

In rats pretreated with famotidine (0.1, 0.3, 1.0 and 3.0 mg/kg), there was no attenuation of the response to sodium nitroprusside infusion, 106 ± 13% to 107 ± 9% (0.1 mg/kg), 111 ± 12% to 106 ± 9% (0.3 mg/kg), 105 ± 15% to 90 ± 8% (1.0 mg/kg) and 102 ± 16% to 113 ± 8% (3.0 mg/kg). These values do not represent a significant reduction from the initial sodium nitroprusside infusion. Famotidine had little effect on mean arterial blood pressure.

4. Discussion

Nitric oxide (NO) is able to cause vasodilation of cranial blood vessels by relaxing smooth muscle tissue through a decrease in intracellular Ca^{2+} (Moncada et al., 1991), and this study shows that NO was able to cause dilation of meningeal blood vessels when injected into the carotid artery. This study also demonstrates that indomethacin can inhibit electrically evoked vasodilation, similar to that found previously with sumatriptan (Williamson et al., 1997a). We observed no effect of either histamine H_1 or histamine H_2 receptor antagonists on NO-induced meningeal vasodilation. The histamine data are consistent with the reported role for NO generation in histamine-induced headache.

Using the intravital microscopy technique, we measured NO-induced changes in meningeal vessel diameter. When NO was injected via the carotid artery, it produced increases in vessel diameter that could be switched on and off in a very similar way to the reproducible effect of histamine infusion we have recently reported. Histamine infusion caused an immediate and reproducible dilation of meningeal blood vessels that could be blocked by histamine H_1 (mepyramine) and histamine H_2 (famotidine) receptor antagonists, as well as by the nitric oxide synthase inhibitor L-nitroarginine methylester (Akerman et al., 2001b). Neurogenic dural vasodilation was not inhibited by histamine H_2 receptor antagonist, but was significantly inhibited by histamine H_1 receptor antagonist. Moreover, we have seen that NO synthase inhibitors can antagonise both neurogenic and calcitonin gene-related peptide-induced meningeal vasodilation (Akerman et al., 2002). During systemic administration, it is believed that NO diffuses into vascular smooth muscle that activates guanylate cyclase, resulting in the formation of cyclic guanosine monophosphate. This decreases intracellular Ca^{2+} and causes a relaxing of the muscle and subsequent dilation of blood vessels (Moncada et al., 1991). This is very similar to human studies where the NO headache was invariably abolished after the infusion of the NO was stopped (Iversen et al., 1989; Olesen et al., 1993, 1994). The delayed response of NO was not studied here, as it seems unlikely to be simply vascular. Administration of histamine H_1 or histamine H_2 receptor antagonists did not inhibit the NO response to meningeal vessel dilation. This indicates that the NO-induced headache is not mediated by histamine. It is doubtful that NO headache depends on the release of histamine to cause dilation of cranial blood vessels (Iversen and Olesen, 1994). It is more likely that histamine-induced headache is actually caused by production of endogenous NO (Iversen et al., 1989; Olesen et al., 1993, 1994).

The effect of sumatriptan on the NO-induced dilation was similar to that found in human studies where the level of dilation was reduced, although not entirely inhibited (Iversen and Olesen, 1996), while sumatriptan alleviated the headache in healthy volunteers. It is believed that sumatriptan inhibits dilation of meningeal vessels by preventing the release of CGRP from trigeminal neurones that innervate the cranial vasculature (Williamson et al., 1997a). Understanding the interaction between CGRP and NO in causing this dilation is therefore going to be crucial in fully characterising the role of sumatriptan or the 5-$HT_{1B/1D}$ receptor agonists play. The effects of the various NO synthase inhibitors on CGRP-induced dilation were not studied here but they may prove crucial in understanding this interaction.

It is possible that NO, as well as causing smooth muscle cells to relax and thus directly dilating cranial blood vessel (Moncada et al., 1991), is also activating trigeminal neurones that release CGRP and cause a dilation of cranial blood vessels during the delayed headache response to NO (Tassorelli et al., 2000). This might explain why sumatriptan is only able to partially inhibit the dilation through the presynaptic inhibition of CGRP release, while the direct effects of NO on the smooth muscle continue to dilate the meningeal blood vessels. In support of this is the fact that, in feline pial arterioles, a chronic unilateral trigeminal ganglionectomy markedly depressed the vasodilator responses to nitrodilators, while on the innervated side application of the CGRP, antagonist human CGRP (8-37) also abolishes the vasodilator response to NO (Wei et al., 1992).

Indomethacin has also been found to prevent and reduce headache occurring with NO infusion (Castellano et al., 1998). It was able to reduce the effects of NO-induced dilation in this study, as well as inhibit the neurogenic dural vasodilatation elicited by electrical stimulation. The implications of the inhibition of neurogenic vasodilation are that it is acting on the trigeminal sensory fibres in some way. Indomethacin inhibits cyclooxygenase activity to prevent the production of prostaglandins (Abdel-Halim et al., 1978), while prostaglandin activity causes the release of both CGRP and NO (Hingtgen et al., 1995; Sakai et al., 1998; Vasko, 1995). Also, electrical stimulation has been shown to induce prostaglandin release in rat hindlimb (Coderre et al., 1990). This leads to the possible conclusion that prostaglandins may be released during the electrical stimulation in the spinal cord, which in part helps cause CGRP and NO production, and leads to the dilation of the cranial vessel. Indomethacin acts to inhibit the production and release of prostaglandins and reduce the level of CGRP and NO production. Complete inhibition was not found in this study, so there is still CGRP release via activation of trigeminal sensory fibres that causes some dilation. It seems unlikely that prostaglandins play a major role in the neurogenic vasodilation, possibly just a contributory factor, as it appears the source of CGRP is from trigeminal neurones.

Flunarizine is a migraine prophylactic (Leone et al., 1991) whose preventative action may involve blockade of Ca^{2+} entry across the cell membrane (Geer et al., 1993). In this study, it did not block either NO-induced or neurogenic vasodilation of dural blood vessels. As was found with the arterial strips, flunarizine is unable to block relaxation and dilation brought about by NO (Ayajiki et al., 1997). Flunarizine was able to inhibit the dilation of arterial strips caused by direct electrical stimulation but not neurogenic vasodilation in dural vessels. It therefore seems likely that flunarizine does not act on trigeminal neurones to prevent CGRP release as its prophylactic action. Although the Ayajiki study and this study show differences with electrically mediated dilation, it is likely that the areas of concern show differing responses to flunarizine, rather than differences between the methods.

In summary, these experiments demonstrate that NO produces dilation of meningeal blood vessels, similar to the way it can produce headache in the clinic. Both sumatriptan and indomethacin inhibited the dilation, with

a likely action via trigeminal neurones. Histamine receptor antagonists were unable to inhibit the NO dilation, indicating that NO alone causes dural vessel dilation. Finally, flunarizine was unable to inhibit both NO-induced and neurogenic vasodilation, indicating that it is unlikely to act on trigeminal neurones.

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