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Neurogenic vasodilation of dural blood vessels is not mediated by cholinergic transmission in the anaesthetised rat☆

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Abstract

Dural vessel dilation induced by activation of trigeminal sensory fibres may be responsible for some component of the migraine attack. The presence in some patients with migraine and cluster headache of clinical features, such as lacrimation, suggests cranial parasympathetic activation and poses the question as to whether neurogenic meningeal dilatation has a cholinergic component. Rats were prepared in order to record on-line the diameter of a middle meningeal artery branch through a closed cranial window using an intravital microscope coupled to a video dimension analyser. Acetylcholine (1 μg, intravenously, i.v.) was administered before and after muscarinic receptor inhibition ($n=5$) with scopolamine (2 mg/kg, i.v.) or nicotinic receptor inhibition ($n=6$) with mecamylamine (4 mg/kg, i.v.). Further vasodilation was induced by electrical stimulation of the cranial window surface before and after muscarinic receptor inhibition with i.v. scopolamine ($n=8$). The mean dural vessel percentage increase caused by acetylcholine stimulation was significantly different before and after muscarinic receptor inhibition ($P=0.045$). Moreover, there was no difference between the post receptor inhibition values and those obtained after vehicle infusion ($P=0.431$). In contrast, no difference was detected in the effect of acetylcholine before and after nicotinic receptor inhibition ($P=0.688$). In the second experiment, where the effect of muscarinic receptor inhibition on the neurogenic dilation model was assessed, no significant difference was demonstrated ($P=0.538$). Cholinergic dilation of the rat dural arteries is mediated by muscarinic receptors, but this mechanism does not play a significant role in the rat dural vessel dilation induced by closed cranial window electrical stimulation.

Keywords: Intravital microscopy; Migraine; Cholinergic

1. Introduction

Migraine is a common neurological condition with a prevalence of 12% on Western populations (Lipton et al., 2001; Rasmussen and Olesen, 1992) that causes substantial disability (Menken et al., 2000) for its most affected sufferers. Cluster headache is less common but no less disabling (Goadsby, 2002). Both conditions are considered to be primarily disorders of the brain with peripheral neurally driven, neurovascular, changes (Goadsby, 2002; Goadsby et al., 2002). A proportion of migraine sufferers present with cranial parasympathetic autonomic activation that is most prominent on the so-called Trigeminal Autonomic Syndromes (Goadsby and Lipton, 1997).

Trigeminovascular activation, or at least the perception of trigeminovascular activation, is likely to be a key component of the pathophysiology of both migraine and cluster headache. Furthermore, many patients report cranial autonomic activation clinically manifest symptoms, such as lacrimation, conjunctival injection, or nasal stuffiness. Cholinergic influences on the trigeminovascular system have not been widely studied. The key components of dural neurogenic inflammation are well understood in terms of antidromic trigeminal activation (Moskowitz and Cutrer, 1993). Parasympathetic activation may play a role in the mechanisms of action of valproate in the neurogenic inflammation model (Cutrer et al., 1997), and in the link between dural inflammation, spreading depression and trigeminal activation (Bolay et al., 2002). It is noteworthy that direct stimulation of the major cranial parasympathetic

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outflow ganglion, the sphenopalatine ganglion, can produce neurogenic dural plasma extravasation (Delepine and Aubineau, 1998). Moreover, stimulation of the major cranial parasympathetic nerve outflow, the VIIth cranial (facial) nerve, is mediated by a nicotinic, hexamethonium-sensitive synapse (Goadsby and Shelley, 1990) in the sphenopalatine ganglion (Goadsby, 1990; Seylaz et al., 1988), and produces increases in cerebral blood flow without concomitant changes in cerebral metabolism (Goadsby, 1989).

In this study, we sought to examine dural neurogenic vasodilator mechanisms in vivo. Williamson and colleagues (1997) 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.

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 mater were clearly visible through the intact skull.

2.2. Intravital microscopy

The cranial window was covered with mineral oil (37 °C) and 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).

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 (NE200 ×, 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 (Williamson et al., 1997). In control experiments, the reproducibility of the neurogenic vasodilator response to four consecutive stimuli was tested in order to determine whether there was any systematic effect over time that might confound the experimental results.

2.3.2. Pharmacological interventions

In the first experiment, acetylcholine (1–10 μg, intravenously, i.v.) was administered three times at 5-min intervals in order to assess the consistency of the acetylcholine-induced vasodilation. Acetylcholine administration was then repeated twice after muscarinic receptor inhibition scopolamine (2 mg/kg, i.v.) (Nakao et al., 1999) or nicotinic receptor inhibition with intravenous mecamylamine (4 mg/kg) (Ruotsalainen et al., 2000). In the second experiment, vasodilation was induced by electric stimulation with a bipolar electrode placed on the surface of the cranial window near the vessel (5 Hz, 1 ms, 10 s) before and after muscarinic receptor inhibition with intravenous scopolamine. Nicotinic receptor inhibition was not used in this second experiment because it did not alter the response to acetylcholine in the first series. Measurements were all commenced 5 min after drug administration.

2.4. Statistical analysis

The effect of acetylcholine infusion and cranial surface electrical stimulation on dural vessel diameter was calculated as the maximum percentage increase in relation to baseline. Student's *t*-test for paired samples was used to compare the means of the values obtained before and after the cholinergic receptor inhibition and differences were considered significant when $P < 0.05$. Summary data are presented as mean ± standard error of the mean (S.E.M.) unless indicated.

3. Results

Animals included in the final analysis had normal cardiovascular parameters. Rats studied in the acetylcholine-induced vasodilation studied weight 369 ± 21 g, while those in the neurogenic vasodilator group weighted 331 ± 17 g. Saline vehicle had no significant effect on dural vessel diameter when tested in animals subsequently receiving acetylcholine in either the mecamylamine (3 ± 2%, $n = 6$) or scopolamine groups (2 ± 1%, $n = 6$).

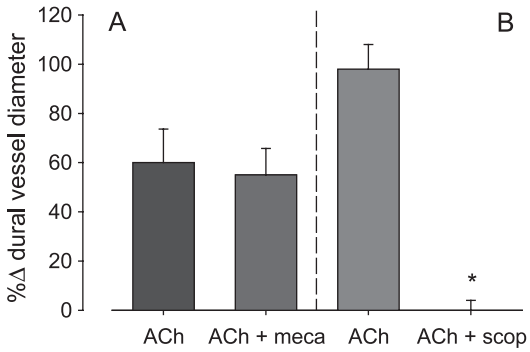

Fig. 1. Dural vessel diameter response to acetylcholine. Acetylcholine (ACh) reproducibly dilates dural vessels in separate populations of animals. There is no effect of the nicotinic blocker mecamylamine (meca-A), while there is a profound inhibition of dural vasodilation with the muscarinic blocker scopolamine (scop-B). *$P<0.05$.

3.1. Acetylcholine-induced dural vasodilation—nicotinic involvement

Acetylcholine induced a dural vessel control vasodilation of $60 \pm 14\%$ ($n=6$). The response to acetylcholine after nicotinic receptor inhibition with mecamlyamine was $55 \pm 11\%$. This was no different from the control response ($t_5=0.43$, $P>0.05$; Fig. 1).

3.2. Acetylcholine-induced dural vasodilation—muscarinic involvement

Acetylcholine induced a dural vessel control vasodilation of $98 \pm 31\%$ ($n=5$). The response to acetylcholine after muscarinic receptor inhibition with scopolamine was $0 \pm 4\%$. There was a significant reduction in the acetylcholine response after muscarinic receptor inhibition ($t_4=2.9$, $P<0.05$; Fig. 1).

3.3. Neurogenic dural vasodilatation—muscarinic involvement

Electrical stimulation of the dura mater resulted in an $81 \pm 10\%$ ($n=9$) increase in dural vessel diameter. The dural vasodilator response after scopolamine was $89 \pm 21\%$, which was no different from the control response ($t_8=-0.5$, $P>0.05$; Fig. 2).

4. Discussion

This study demonstrates that whereas acetylcholine induces a potent dilator response in the dural circulation that is blocked by the muscarinic receptor antagonist scopolamine, and not the nicotinic receptor antagonist mecamylamine, neurogenic dural vasodilation has neither a muscarinic nor nicotinic component.

Primary headache syndromes share the same pain expression systems, involving cranial vessels and their parasympathetic innervation (May and Goadsby, 1999). The relevance of cranial vasodilation to the pathophysiology of primary headaches has been well demonstrated by the effectiveness of the triptans, serotonin 5-$HT_{1B/1D}$ receptor agonists, in treating acute migraine (Ferrari et al., 2002) and cluster headaches (Matharu et al., 2003). Vasodilation observed in primary headache is elicited by antidromic transmitter release of calcitonin gene-related peptide from trigeminal fibres and by augmented the VIIth cranial nerve parasympathetic outflow (Edvinsson and Goadsby, 1995). Vasoactive intestinal peptide, one of the transmitters present in cranial vasomotor parasympathetic fibres (Edvinsson and Ekman, 1984; Hara et al., 1989), was found to be increased in acute cluster headache (Fanciullacci et al., 1995; Goadsby and Edvinsson, 1994) and paroxysmal hemicrania (Goadsby and Edvinsson, 1996). This is not observed robustly in migraine (Goadsby et al., 1990), correlating with the lower incidence of autonomic signs in migraine. On the other hand, the presence of unilateral autonomic symptoms was associated to more severe and unilateral pain in a series of 177 migraineurs (Barbanti et al., 2002), and to exacerbations, but not to baseline headache, in hemicrania continua patients (Peres et al., 2001). The changes are consistent with a view of trigeminal-autonomic reflex activation being determined by the severity of pain integrated by the primary headache type and its interaction with that reflex (Goadsby et al., 2001).

The capacity of lidocaine blockade of the pterygopalatine (sphenopalatine) ganglion to abort acute attacks of migraine (Maizels and Geiger, 1999) and cluster headache (Costa et al., 2000; Kitrelle et al., 1985; Kudrow and Kudrow, 1995) argues for its involvement in both disorders. Markers for acetylcholine synthesis are found in the sphenopalatine ganglion of many species (Hara et al., 1989; Uddman et al., 1999), while stimulation of the sphenopalatine ganglion in rat (Seylaz et al., 1988; Suzuki et al., 1990) and cat (Goadsby, 1990) increases cerebral blood flow. Stimulation of the VIIth cranial nerve leads to local release of vasoactive intestinal polypeptide (Goadsby and Shelley, 1990), and can be blocked by vasoactive intestinal polypeptide antagonism

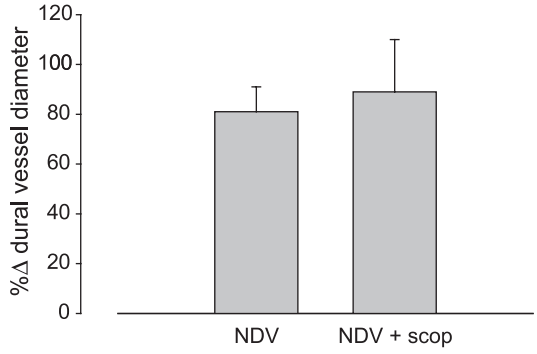

Fig. 2. Neurogenic dural vasodilator response. Stimulation of the dura mater results in a potent, reproducible dilation of the dural vessels (neurogenic dural vasodilation—NDV), that is unaffected by muscarinic receptor inhibition by scopolamine (scop).

(Goadsby and Macdonald, 1985). In keeping with the new data, there is no significant effect of cholinergic blockade on VIIth nerve-induced increases in cerebral blood flow (Goadsby, 1991).

Direct measurement dural blood vessel diameter using the intravital method in the anesthetised rat through a closed cranial window has documented dural vasodilation induced by calcitonin gene-related peptide (CGRP), substance P and neurokinin A (Williamson et al., 1997). The combination of short duration, low-intensity electric transcranial stimulation of the trigeminal afferents and this direct imaging technique has resulted in considerable advances in characterising trigeminovascular neural vasodilation (Akerman et al., 2002a; De Vries et al., 1999). The method's strengths are in reproducibility and no requirement to remove the bone affording protection of the local dural environment. The method's chief limitation is the need for systemic administration of drugs. A complementary approach has been that of Strecker et al. (2002) in studying dural vessels with laser Doppler, with the disadvantage of requiring the bone to be disturbed. The results are generally similar (Akerman et al., 2002b; Dux et al., 2000).

In summary, the study has shown that dural vessels contain functional cholinergic muscarinic receptors that medicate vasodilation associated with administration of acetylcholine, but no population of nicotinic receptors. In contrast, neurogenic dural vasodilation is unaffected by cholinergic muscarinic receptor inhibition, and this cholinergic mechanisms are unlikely to play a major role in the neurogenic modulation of dural–vascular afferents. Understanding the contribution of the trigeminal–cranial autonomic system in terms of its physiology and pharmacology is essential to building a complete picture of the anatomy and physiology of primary headaches.

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