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# Retinal plasma extravasation in animals but not in humans: implications for the pathophysiology of migraine

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## Summary

**High-intensity electrical stimulation of the trigeminal ganglion is accompanied by mast cell degranulation, vasodilatation, increased endothelial permeability and leakage of albumin from postcapillary venules within the dura mater. Overall, the histological appearance suggests an evolving sterile inflammatory response. This neurogenic inflammation within the meninges has been suggested as a model to explain the pain in migraine and cluster headache, and has been used to characterize the pharmacology of anti-migraine compounds. Using the rat model of neurogenic inflammation, the albumin extravasation ratio (stimulated : unstimulated side) in vehicle-treated animals in the dura and retina was 1.60 ± 0.11 and 1.76 ± 0.18, respectively ($n$ = 10; values are mean ± SEM). Pretreatment with sumatriptan ($n$ = 9) produced a highly significant reduction in the ratio of extravasation within the dura to 1.10 ± 0.06 ($P$ = 0.002) and in the retina to 0.96 ± 0.06 ($P$ ± 0.001), as did the neurokinin-1 receptor antagonist RP 67580 ($n$ = 12) in the dura (1.04 ± 0.11, $P$ = 0.002) and retina (1.08 ± 0.06, $P$ = 0.001). These data demonstrate increased endothelial permeability and leakage of albumin not only in the dura but also in the retina. In a second stage we investigated possible extravasation in the human retina in acute migraine ($n$ = 8) and cluster headache ($n$ = 5) using fluorescein or indocyanine angiography. No increased endothelial permeability or leakage of dye could be found in the human retinal or choroidal vessels during headache attacks or in the headache-free interval in persons suffering from both migraine and cluster headache. These data raise the possibility that neurogenic inflammation is not a major factor in headache attacks in migraine or cluster headache.**

**Keywords**: migraine attack; neurogenic inflammation; trigeminovascular system; plasma extravasation; fluorescein angiography

## Introduction

The underlying pathophysiology of migraine remains unclear. Scientific investigation is difficult because migraine can only be studied directly in humans. Traditionally, migraine has been linked to pathological changes in the diameter of cranial blood vessels. As early as 1931, Rodella reported widening of the retinal vessels, which he observed by examining the fundi of his patients during the headache. Three years later Critchley (1934) also noted engorgement and tenderness of the temporal vessels during headache, with congestion of the conjunctival and retinal vessels. He concluded that these findings supported a vascular origin of migraine, although later in life Critchley no longer held that view (personal communication to P.J.G.). Wolff (1963) suggested that diameter changes in extracranial and most likely intracranial arteries were the cause of headache. This hypothesis seemed to explain the therapeutic benefit of substances with vasoconstrictor effects, such as ergotamine, dihydroergotamine and sumatriptan, although other substances with even stronger vasoconstrictor activity, such as angiotensin and noradrenaline, had no effect. The suggestion that the dura mater and its small vessels is an important source of headache pain, indeed resulting in pain when electrically or mechanically stimulated (Cushing, 1908; Ray and Wolff, 1940), led to the hypothesis that additional mechanisms may be involved (Strassmann *et al.*, 1986; Moskowitz, 1993).

In 1987 Moskowitz and her co-authors (Markowitz *et al.*,

in four cluster patients was triggered using inhalation of nitroglycerine during their bout; one patient suffered from a spontaneous attack. The headache attack in the eight migraine patients was untreated and spontaneous. Excluded were patients who received prophylactic treatment or other vasoactive drugs, patients with drug or analgesic abuse and patients who suffered from chronic tension-type headache or any other known illness other than migraine. All patients were studied within 6 h of the onset of untreated headache symptoms.

Each patient had two examinations: (i) during the acute attack and (ii) during the headache-free interval 3 days to 4 months later.

Each patient's visual acuity was tested, then examination by ophthalmoscopy and slit lamp was performed; the patient was then subjected to fluorescein or indocyanine angiography. In all patients the eye on the side ipsilateral to the headache side was examined. A 5 ml intravenous injection (right brachial vein) of 10% sodium fluorescein (four patients) or 25 mg indocyanine green dye, dissolved in 5 ml aqueous solvent (10 patients) (Table 1) was immediately followed by angiography (Flower, 1973; Wessing, 1975; Bischoff and Flower, 1985; Nielsen, 1985; Panzardi *et al.*, 1992; Lim and Flower, 1995), using a modified fundus camera and a TV system for image reception. Angiographies were recorded on videotape and images were simultaneously digitized and saved on disc. Two migraine patients received both treatments. The late pictures were taken 10–12 min after this procedure. The complete procedure was repeated in the headache-free interval. The immediate angiogram of the headache attack was then compared with the late pictures to detect vascular leakage, as well as with the early and late angiograms of the headache-free interval. The person reporting the angiograms was different from the person involved in the actual study and was blinded to the clinical data.

## Results

### *Animal study*

Following electrical stimulation of the trigeminal ganglion in vehicle-treated animals, the extravasation ratio in dura and retina was 1.60 ± 0.11 and 1.76 ± 0.18 respectively ($n$ = 10; values are mean ± SEM). Pretreatment with sumatriptan ($n$ = 9) produced a highly significant reduction in the ratio of extravasation within the dura to 1.10 ± 0.06 ($P$ = 0.002) and in the retina to 0.96 ± 0.06 ($P$ = 0.001), as did RP 67580 ($n$ = 12) in the dura (1.04 ± 0.11, $P$ = 0.002) and retina (1.08 ± 0.06, $P$ = 0.001) (Fig. 1). The extravasation ratio in the facial tissues in the control group was 4.00 ± 0.51 (eyelid), 3.57 ± 0.79 (lip) and 1.34 ± 0.26 (conjunctiva). Sumatriptan had no significant effects on the extravasation ratio in the facial tissues but pretreatment with RP 67580 diminished the ratio in the eyelid to 1.71 ± 0.21 ($P$ = 0.001), in the lip to 1.34 ± 0.15 ($P$ = 0.006) and in the conjunctiva to 1.24 ± 0.08 (not significant).

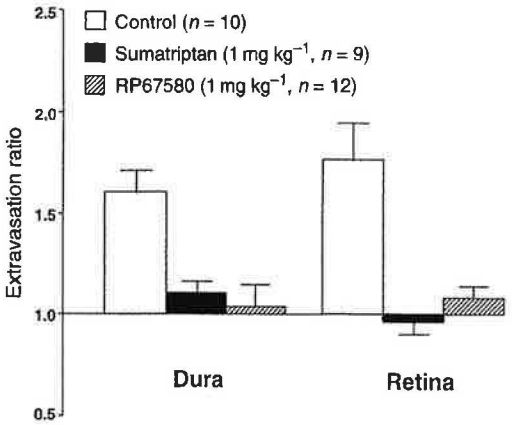


**Fig. 1** Extravasation ratio in the retina and dura following unilateral electric stimulation of the trigeminal ganglion (white bars) and after pretreatment with sumatriptan (black bars) or RP67580 (hatched bars).

**Table 2** *Percentage change of vital parameters from baseline (before unilateral ganglion stimulation) in experimental rats* (n = *3)*

| | During stimulation | 1 min after stimulation | 5 min after stimulation |
|---|---|---|---|
| $pCO_2$ | –4.92% | –1.37% | 0.13% |
| $pO_2$ | 31.93% | 15.12% | 18.70% |
| pH | 0.38% | –0.22% | –0.28% |
| Blood pressure | 44.49% | 16.80% | 11.92% |
| Heart rate | 18.29% | 10.75% | 7.38% |

In three additional animals without pretreatment, the arterial blood pressure and heart rate showed a moderate increase during ganglion stimulation. The arterial $pO_2$ also increased whereas $pCO_2$ and pH moderately decreased, indicating mild hyperventilation (Table 2).

### *Human study*

No significant changes in retina background and especially no leakage of plasma extravasation markers (i.e. fluorescein or indocyanine green) from the retinal or choroidal vessels were seen in either the acute migraine attack or the headache-free interval. This held true immediately after injection of the dye as well as in the late pictures taken 10–12 min after injection of the bolus (Fig. 2A–D] All patients had a normal retinal angiogram. In summary, no increased endothelial permeability or leakage of dye could be detected in any of the 13 patients.

## Discussion

In 1987 Moskowitz and co-authors introduced an animal model of neurogenic inflammation in the dura mater where plasma protein extravasation, vasodilatation, increased endothelial permeability and mast cell degranulation were

effective in blocking dural plasma extravasation in this animal model. In humans, however, we failed to show any extravasation in the acute headache attacks of migraine and cluster headache patients.

The methods used in the animal and human studies are compatible in that indocyanine green dye binds to albumin (Panzardi *et al.*, 1992; Lim and Flower, 1995) and fluorescein binds incompletely to albumin and to a considerable extent to even smaller proteins (Hodge and Dollery, 1964; Wessing, 1968), and should be extravasated into the tissue if extravasation takes place in the human retina during the acute migraine or cluster attack (Lange and Boyd, 1944; Panzardi *et al.*, 1992). If the model of neurogenic inflammation is transferable to humans, proteins at least as large as albumin (which is the marker for extravasation in this model) should be extravasated. We cannot exclude the possibility of a different physiology or trigeminal innervation and sensitivity of the dura and retina in humans compared with animals. Several aspects of the model of trigeminal-induced plasma extravasation clarify its limitations with regard to migraine. Serotonin does not block plasma extravasation following trigeminal ganglion stimulation, although it is effective in humans (Kimball *et al.*, 1960; Lance *et al.*, 1967). This issue is confused by the potentially proinflammatory effects of serotonin at the 5-$HT_{2A}$ receptor (Verheyen *et al.*, 1987; Bryant *et al.*, 1996). Neurologists would not generally consider indomethacin or valproate as first-line abortive agents in migraine yet they are highly effective in neurogenic inflammation. Furthermore, avitriptan, another 5-$HT_{1B/1D}$ agonist that is clearly an effective anti-migraine agent (Couch *et al.*, 1996) of similar efficacy to sumatriptan, is much less potent than sumatriptan in the neurogenic plasma extravasation model (Yocca *et al.*, 1997). Moreover, plasma extravasation following trigeminal ganglion stimulation can be blocked by pretreatment with sumatriptan (Buzzi *et al.*, 1991*b*), while pretreatment of headache in patients using sumatriptan during the aura does not block headache (Bates, 1993). Consistent with these observations, and with our new data, plasma extravasation has not been observed in humans during migraine (Nissila *et al.*, 1996).

Although our data indicate that the vital parameters, such as blood pressure, heart rate and blood gases, show only a moderate change during stimulation, it is important to consider that lowering a bipolar electrode directly through the brain and then stimulating the trigeminal ganglion with high current is non-physiological and may well produce a whole range of changes within the trigeminovascular system. Possible factors other than neurogenic inflammation involved in human migraine attacks are hyperexcitability of the cortex (Welch *et al.*, 1993), disturbances of the blood–brain barrier (Kaube *et al.*, 1993), abnormal vascular reactivity and release of neurotransmitters, such as calcitonin gene-related peptide and substance P (Goadsby and Edvinsson, 1993). The source of the migraine headache is still not clear. Vascular dilatation alone is unlikely to explain it. Activation of trigeminovascular neurons causes the release of neuropeptides. The 5-HT1 receptors on these trigeminovascular nerves may modulate the central transmission of nociceptive inputs (Feniuk *et al.*, 1979; Connor and O'Shaughenessy, 1993; Kaube *et al.*, 1993; Hoskin *et al.*, 1996). The trigeminovascular system appears to play a pivotal role as the substrate for craniovascular nociception (Goadsby and Edvinsson, 1993, 1994), although what excites the perivascular nociceptors in the first place remains to be established.

In conclusion, these new data, taken together with the facts that certain substances are potent inhibitors of neurogenic extravasation but ineffective in human migraine and that substances effective in migraine are relatively ineffective in the model of neurogenic extravasation, indicate that the model of inflammatory neurogenic activation of C-fibres might not be an appropriate model to elucidate the clinical efficacy of the entire range of novel substances in migraine. The model as it is currently employed seems to select substances that are active against extravasation but may not necessarily prove to have anti-migraine activity. Our data raise the possibility that neurogenic inflammation is not present or, if present, forms a role that is not sufficient by itself to produce pain in migraine or cluster headache since its blockade does not necessarily relieve headache. The data support the view that other fundamental processes, most likely in the central nervous system, are necessary and key to the pathophysiology of an acute migraine attack.

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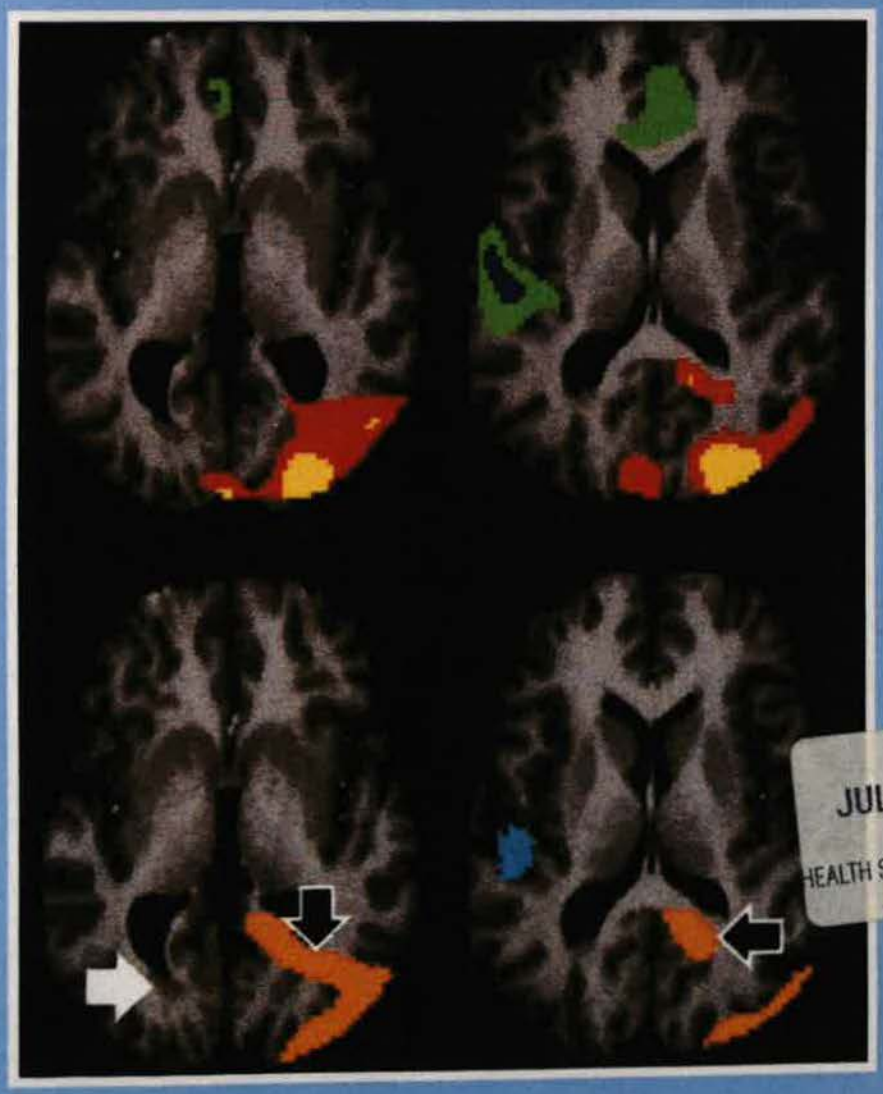



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