SHORT REPORT

Decreased CGRP, but preserved Trk A immunoreactivity in nerve fibres in inflamed human superficial temporal arteries

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Abstract

The peptidergic sensory innervation of cranial blood vessels may play an important part in vascular head pain. The neuropeptides calcitonin gene-related peptide (CGRP) and substance P in sensory fibres are dependent on nerve growth factor (NGF) produced by the blood vessels, and when released from nerve terminals mediate neurogenic inflammation. NGF is increased in inflamed tissues, and acts via its high affinity receptor trk A on nociceptor fibres to produce hyperalgesia. CGRP and trk A immunoreactive nerve fibres have therefore been studied, for the first time, in inflamed (n=7) and non-inflamed (n=10) temporal arteries biopsied from patients with headache and suspected giant cell arteritis. CGRP immunoreactivity was markedly decreased to absent in adventitial nerve fibres in inflamed regions of vessels, which may reflect secretion from nerve terminals, as CGRP immunoreactivity could still be seen in nerve trunks in periadventitial tissue. Trk A immunoreactive nerve fibres were found in a similar distribution to CGRP containing nerve fibres in non-inflamed vessels, and the trk A immunoreactivity was virtually unchanged in inflamed vessels. The evidence supports a role for NGF related mechanisms in inflammatory vascular head pain. Anti-NGF or anti-trk A agents may represent novel analgesics in this condition.

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The cranial vasculature derives a rich supply of nociceptor fibres from the trigeminal system, containing the neuropeptides calcitonin gene related peptide (CGRP) and substance P.¹⁻⁴ It has been proposed that activation of these nerves and release of sensory neuropeptides from their peripheral terminals leads to neurogenic inflammation, which may potentiate vascular headache.⁵⁻⁷

The sensitivity of nociceptor fibres and levels of expression of their sensory neuropeptides are dependent on nerve growth factor (NGF).^{8,9} NGF is produced by blood vessels^{10,11} and acts via its high affinity receptor trk A on nociceptor fibres.^{12,13} We have marshalled evidence in support of the hypothesis that NGF concentrations may regulate the presentation of vascular head pain, particularly in inflammatory states.¹⁴ We have demonstrated the presence of NGF in postmortem human cerebral blood vessels,¹¹ and NGF concentrations are increased in inflamed tissues.^{14,15}

The superficial temporal artery provides a tissue model in humans to study the relation of vascular inflammation to NGF related pain mechanisms, as biopsy specimens may be obtained from patients suspected of giant cell arteritis. Giant cell arteritis is an inflammatory condition in which headache usually predominates, and biopsy of the superficial temporal artery is performed to confirm the diagnosis.

In this study, we have shown trk A immunoreactive nerve fibres in blood vessels for the first time, and compared their distribution in inflamed and uninfamed superficial temporal arteries to CGRP immunoreactivity.

Methods

Superficial temporal artery biopsies were obtained from 17 patients suspected of having giant cell arteritis. All patients were middle aged or elderly (mean age 72 years, range 41-87 years) with headache, and some had other symptoms including lethargy and weight loss. Prednisolone had been commenced in all cases in doses of 30 mg-60 mg daily some days before biopsy. The specimens were transported in moist gauze, and kept cool on wet ice. Seven biopsies showed histological evidence of active arteritis with cellular infiltrate, giant cells, destruction of the elastic lamina in all cases, and almost complete occlusion of the artery lumen. The remaining 10 arteries were of normal appearance, with only age related changes. A separate portion of each biopsy was examined independently by the pathology department of the hospital where it was performed, with the same results. Clinical management

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and follow up of the cases have all been in accord with the biopsy diagnosis.

For immunostaining studies, cryostat frozen sections (8 μ m) were thaw mounted on to

Figure 1 (A) Immunoreactive CGRP nerve fibres in a small adventitial nerve trunk (arrow) from an uninflamed human temporal artery; note that only a small proportion of fibres in the nerve are positive for this peptide. (B), (C) Trk A immunoreactivity in perivascular nerves in uninflamed human temporal artery. Numerous fibres are present at the border of the muscular media and also in an adjacent adventitial nerve trunk. t=nerve trunk; l=lumen of artery; scale bar=100 μ m.

poly-L-lysine coated glass slides and fixed for 1 hour at room temperature in 4% paraformaldehyde. Endogenous peroxidase was inhibited by immersing the sections in H_2O_2 (0.3% in methanol) for 30 minutes, followed by incubation for 10 minutes with either normal goat serum (1:30) or normal horse serum (1:30). Sections were subsequently incubated with primary antibody (anti-CGRP 1:5,000 (Hammersmith Hospital, UK), or anti-trk A 1:400 (Genentech Inc, USA)) overnight at room temperature. Immunoprecipitate was visualised using an enhanced avidin-biotin peroxidase method (ABC, Vecta Labs, USA). To demonstrate specificity, trk A immunoreactivity was preabsorbed with trk A-IgG protein (Genentech Inc, USA) at a concentration of between 0.0014 and 0.00014 mg/ml, but not with trk B-IgG protein (up to 0.0038 mg/ml).

Immunoreactive nerve profiles in tissue sections assessed as follows by light microscopy: abundant nerve fibres scored as 3, moderate number of fibres as 2, few fibres as 1, and no fibres as 0.

Results

CGRP immunoreactivity was seen in a few delicate nerve fibres located at the adventitia/media border of the artery, and also in small nerve trunks running within the adventitia (fig 1A). The staining pattern had a varicose appearance, consistent with axonal immunoreactivity. Only a small proportion of nerve fibres associated with the vessel were immunoreactive for CGRP, as can be seen in the photomicrograph. These findings are in accord with previous publications.¹⁻⁴ In the inflamed tissue, no CGRP immunoreactive nerve fibres were seen at the adventitia/media border. However, CGRP immunoreactivity was still detected in small nerve trunks running within the connective tissue surrounding the artery.

Trk A immunoreactivity was demonstrated in numerous small nerve fibres, distributed circumferentially at the adventitia/media border, and also within small nerve trunks associated with the vessel in the surrounding connective tissue (fig 1B and C). Trk A immunoreactive fibres were virtually unchanged in the inflamed vessels, both in the adventitia and in the surrounding connective tissue, although adventitial immunoreactivity was not as intense as in non-inflamed vessels.

The semiquantitative analysis showed highly significant reduction of CGRP immunoreactive nerve fibre profiles in inflamed vessels when compared with uninflamed vessels ($p=0.0016$, Student's unpaired t test), but no significant change of trk A immunoreactive nerve fibre profiles in the same specimens (fig 2).

Discussion

Although substance P, CGRP, and other neuropeptides have been previously demonstrated within the adventitia of blood vessel walls, including those of the superficial temporal artery,³⁻⁴ we have shown for the first time the presence of the high affinity nerve growth factor receptor trk A within nerves in blood

Figure 2 Comparison of immunoreactive nerve in human inflamed temporal artery ($n=7$) and control uninflamed temporal artery ($n=10$); (A) CGRP; (B) Trk A. There is a significant reduction in the overall immunoreactivity for CGRP ($*p=0.0016$, Student's unpaired t test) in the inflamed specimens. There is little change in the same parameter for Trk A.

vessels. Trk A immunoreactive nerve fibres were more abundant than CGRP containing fibres, as expected, as many of the trk A positive fibres are sympathetic in origin, and do not express CGRP, whereas others are sensory in origin.

Inflammation seemed to markedly reduce CGRP immunoreactivity in nerve fibres, although weak immunoreactivity was still seen in nerve trunks present in the periadventitial tissue, and trk A immunoreactivity was virtually unchanged. The reduced CGRP immunoreactivity may thus reflect secretion of the peptide from nerve terminals in inflamed tissue, and possibly from central terminals, although there are other possible interpretations, such as interference of the CGRP immunostaining by inflammation. Similar changes of CGRP in nerve terminals have been described in inflamed rabbit bowel.¹⁶ There is evidence of related mechanisms in migraine. Goadsby *et al*^{6,17} have measured increased concentrations of CGRP and substance P in the ipsilateral external jugular vein in patients with migraine during the headache. Biopsies of tender superficial temporal arteries in migraine may show oedema, attributed to local release of substance P, and in cluster headache show increased mast cells during headache free intervals⁷; NGF sensitises nociceptor fibres directly, enhances neurogenic inflammation via substance P and CGRP concentrations, and is associated with increased numbers of mast cells.^{8,13,18}

Modulation of NGF activity may provide a new approach to prevent and treat vascular headaches. Corticosteroids help inflammatory vascular headache, as in temporal arteritis, and also reduce NGF synthesis in cultured cells.¹⁹ Although our patients had received corticosteroids before biopsy, it could be argued that the changes we describe would have been even more pronounced without this treatment. Anti-NGF agents, such as NGF sequestering molecules,¹⁸ or anti-trk A agents, may also prove to be useful therapeutic strategies in vascular head pain.

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