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

For immunostaining studies, cryostat frozen sections (8 µm) were thaw mounted on to 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 H₂O₂ (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. 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, we have shown for the first time the presence of the high affinity nerve growth factor receptor trk A within nerves in blood
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 al17 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 sen-

tises nociceptor fibres directly, enhances neuro-
genic inflammation via substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in pain.

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 cortico-

steroids 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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