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Corticotropin releasing factor receptor expression in painful human dental pulp

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ABSTRACT

Background: Our objective was to correlate the presence of symptoms and dental pulp injury with the amount of a subtype of corticotropin releasing factor receptor (CRF-R) in symptomatic and asymptomatic human teeth. We hypothesized that patients diagnosed with irreversible pulpitis have increased levels of CRF-R.
Materials and methods: Dental history, diagnosis and radiographs were obtained from treatment records following extractions. Teeth were diagnosed as asymptomatic or symptomatic demonstrated by clinical and radiographic evaluation. Tissue sections from tooth pulp were immunoreacted with antibodies directed against CRF receptor 2 (CRF-R2) and neurofilament protein and examined to correlate CRF-R expression with pulpal diagnosis.
Results: Our results indicated that symptomatic pulps demonstrated significantly greater expression of CRF-R2. The increased expression was localized on distinct cellular profiles throughout the pulp and was not directly correlated with neurofilament expression.
Conclusions: Our findings suggest that the analgesic effects of endogenously produced CRF may be enhanced via upregulation of CRF-R2 expression, and may explain the occurrence of reduced pain symptoms in some patients with irreversible pulpitis. The application of CRF-R agonists may be a feasible strategy in reducing pain associated with irreversible pulpitis.

1. Introduction

Corticotropin-releasing factor (CRF) is a 41-amino acid residue peptide that modulates endocrine, autonomic, and behavioural activity in response to stress, as well as a range of peripheral activities.[1,2] CRF is a critical coordinator of the hypothalamic-pituitary-adrenal (HPA) axis, and binds to CRF receptors (CRF-Rs) of the anterior pituitary, thereby stimulating secretion of adrenocorticotropic hormone (ACTH) and β-endorphin (END)[3] an important physiological modulator of nociception.[4] ACTH stimulates secretion of cortisol, an inflammatory glucocorticoid, from the adrenal gland.[5,6] CRF plays hypothesized, diverse roles in homeostasis, such as regulating circadian rhythm,[7] labour and parturition[8] and modulating stress-related behaviours,[9,10] including maternal care in rodents.[11] Since the discovery of CRF, the presence of CRF-R1 and CRF-R2 in humans has been demonstrated in the central nervous system (CNS) as well as peripherally in skin[12] and blood vessels.[13] In peripherally inflamed tissues, activation of CRF-Rs on immune cells stimulates the release of END that subsequently interacts with opioid receptors on nerve endings and elicits local analgesia. The process, known as opioid-mediated pain inhibition,[14,15] increases the analgesic effect of acupuncture in a carageenan model of inflammation[16] and significantly reduces postoperative pain after

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arthroscopic knee surgery.[17] Increased numbers of END-containing immune cells, including T-lymphocytes, were found in inflamed rat pulp tissue after mechanical exposure.[18] CRF-R expression is increased in inflamed rat dental pulps,[19] however CRF-Rs in human dental pulp have yet to be reported. Peripheral nerve endings within human dental pulp contain both δ- and μ-opioid receptors[20,21] that may be activated by CRF mediated processes.

Because of the prominent role of CRF in peripheral antinociception, we hypothesize that CRF-R2 may play a similar role in human dental pulp and is upregulated in inflammatory pain states. To test our hypothesis, we compared levels of CRF-R2 immunoreactivity in healthy, asymptomatic pulp tissue with irreversibly inflamed symptomatic pulp tissue in extracted human teeth using fluorescent immunocytochemistry.

2. Materials and methods

2.1. Subjects

Our study was approved by the Biomedical Institutional Review Boards and the informed consent of all participants was obtained. Healthy, normal molars and molars diagnosed with symptomatic irreversible pulpitis were extracted according to the treatment plan for each patient ($n = 16$ for both groups). Teeth with irreversible pulpitis were diagnosed based on clinical presentation of spontaneous pain, and pain upon thermal stimulation (heightened and/or lingering response to cold), and/or heightened response to percussion due to pulpal inflammation. Asymptomatic teeth were devoid of any of the above symptoms and had no indication of caries. All teeth were permanent teeth with fully developed apices and free of visible cracks.

2.2. Tissue processing

Specimens were processed for immunohistochemical analysis according to previously described methods.[19] Extracted teeth were collected in freshly prepared 4% paraformaldehyde in 0.15 M phosphate-buffered saline (PBS) at 4 °C for not more than 7 days. Teeth were scored longitudinally, using a carbide bur in a high speed handpiece, taking care not to access the pulp and split using blunt force along the score marks, pulp tissue was removed, placed in 4% paraformaldehyde for 1 h at room temperature and transferred to 30% sucrose in 0.15 mol/L PBS overnight at 4 °C. Pulp tissue was embedded in Tissue-Tek (Fisher Scientific, Pittsburgh, PA), frozen, and 12-μm sections were collected on SuperFrost Plus glass slides (Fisher Scientific). Tissue from one specimen was lost during processing yielding 15 normal tissue specimens and 16 irreversible pulpitis specimens submitted for immunocytochemical analysis.

2.3. Immunocytochemistry

Tissue sections were rinsed (three times for 10 min each in 0.15 M PBS) and incubated in 6% normal donkey serum with 0.4% Triton X-100 in 0.15 M PBS for 1 h in order to block non-specific antibody immunoreactivity.

Sections were then incubated with antibodies directed against human CRF-R2 (rabbit host, polyclonal, at 1:1000 final concentration; Abcam), and human neurofilament protein (chicken host, polyclonal, at 1:20,000 concentration; Chemicon). Sections were incubated for 72 h at 4 °C. To account for non-specific immunoreactivity, representative sections from each pulp were processed identically either without primary antibody or with primary antibody that had been preadsorbed with the corresponding antigenic peptide (negative controls). Control images demonstrated lower immunofluorescence when collected in the same manner as the sections incubated in primary antibody (Fig. 2).

After incubation, all specimens (including negative controls) were rinsed (three times in 0.15 M PBS for 10 min each) and incubated in secondary antibodies for 1 h in the dark. The secondary antibodies, AlexaFlour-488 conjugated donkey anti-chicken and AlexaFluor-555 conjugated donkey anti-rabbit were used to reveal distribution of tissue-bound neurofilament and CRF-R2 antibodies, respectively (all final concentrations were 1:500; Invitrogen, Carlsbad, CA) in 0.15 M PBS and 0.1% Triton-X-100. Slides were rinsed (three times in 0.15 mol/L PBS for 10 min each) and coverslipped with ProLong Gold antifade reagent (Invitrogen).

2.4. Data collection and statistical analysis

Individual 12 μm sections were viewed with a FluoView FV300 confocal laser scanning microscope (Olympus, Center Valley, PA) and appropriate laser lines to visualize CRF-R2 and neurofilament. In all teeth, a field of interest in the pulp was selected by a blinded examiner using only neurofilament immunofluorescence to visualize nerve fibres. A region of interest within the field, approximately 480,000 μm^3 (200 μm long × 200 μm wide × 12 μm section thickness) was used to capture stacks of optical sections at 1 μm intervals for both CRF-R2 and neurofilament. Image capture and analysis were performed with the same microscope parameters for each specimen, allowing data from each sample to be directly compared with other samples. The ratio of immunofluorescence of the sample to background fluorescence was calculated and is expressed as the 'signal-to-noise ratio'. Background fluorescence was defined as fluorescence of a tissue section processed identically as the sample of interest, but without the addition of primary antibody. Distributions were tested for normality with the Shapiro Wilk W-test. Differences in CRF-R2 fluorescence scores between symptomatic and asymptomatic cases were tested with the Wilcoxon test. For all comparisons, α was set at 0.05.

3. Results

3.1. Neurofilament

We observed qualitative differences in the amount and distribution of nerve endings in the specimens by labelling them with antibodies directed against neurofilament proteins. In general, there was an increased amount of nerve fibres and branching among the symptomatic (irreversible pulpitis) compared to the asymptomatic (healthy) specimens (Fig. 1).