THE NEUROCHEMICAL RESPONSE IN FORELIMB TENDONS IN A MODEL OF UPPER EXTREMITY WORK-RELATED MUSCULOSKELETAL DISORDER

*Fedorkycz, J M; **Barr, A E; **Amin, M; **Barbe, M F
*Temple University and Drexel University, Philadelphia, PA
+**Temple University, Philadelphia, PA

mbarbe@temple.edu

ABSTRACT INTRODUCTION:

Despite increased awareness of ergonomic risk factors in the workplace, incidence and costs of work-related musculoskeletal disorders (WMSDs) are high. Tendinopathies are associated with repetitive movement, including active muscle contractions and stretching over bony surfaces, with and without force. Occurrence increases with age and the degree of exposure to forceful repetitive movement.

Degenerative changes, named tendinosis, have been observed in both patients with upper extremity tendinopathies and in animal models. Despite the absence of inflammation in tendinosis tissue, tendinopathies still present with clinical pain. Recent studies on patients with chronic tennis elbow have identified the presence of neurochemicals in the ECRB tendon, the muscle most implicated in tennis elbow. Increased presence of glutamate, peptide, and N-methyl-D-aspartate receptor (NMDA) receptor (r1) has been observed in patients with tennis elbow. Glutamate activates the NMDA receptor. The presence of significant levels of substance P (SP), glutamate and calcitonin gene-related peptide (CGRP) within tendinosis tissue may provide an alternative mechanism to inflammation for pain mediation in chronic tendinopathies.

Our goal for this study was to examine the presence and timing of SP, CGRP and the NMDA receptor (r1) using immunohistochemical techniques in forelimb tendons of rats performing a repetitive reaching and grasping task using a rat model of WMSD developed in this laboratory.

METHODS:

Twenty five young adult female Sprague-Dawley rats were used. Twelve rats performed a high repetition high force task (HRHF; 60% maximum grip) in which grasping a lever occurred at a target rate of 4 reaches/min, 2 hrs/day, 3 days/week for up to 12 wks. Eight rats performed a low repetition low force task (LRLF) in which they retrieved a 45mg food pellet at a target rate of 2 reaches/min, 2 hrs/day, 3 days/week for up to 12 wks. The remaining rats were controls.

All experiments were approved by the Temple University Institutional Animal Care and Use Committee in compliance with NIH guidelines for the humane care and use of laboratory animals. Studies were conducted on female Sprague-Dawley rats (285-310 g), which were housed in the central animal facility in separate cages in a 12 hour light/dark cycle with free access to water. Following euthanasia (Nembutal, 120 mg/kg body weight), forelimb tissues were collected and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PBS) for 30 minutes, washed, then incubated for 20 minutes in either 4% milk/PBS (SP, CGRP) or 4% goat serum (NMDAR1). Tissues were incubated overnight at 24°C in 0.1 M phosphate buffered saline (PBS) for 30 minutes, washed, then incubated for 20 minutes in either 4% milk/PBS (SP, CGRP) or 4% goat serum (NMDAR1). Tissues were incubated overnight at 24°C with either rabbit anti-SP antibody (Chemicon, 1:500), anti-CGRP antibody (Chemicon, 1:100) or mouse anti-NMDAR1 (BD PharmMingen, 1:250). Following tissue washing in PBS, the secondary antibody was applied for 2 hours using goat anti-rabbit HRP (Jackson ImmunoResearch, 1:100) for SP and CGRP and goat anti-mouse HRP (Jackson ImmunoResearch, 1:100) for NMDAR1. DAB reaction was used to visualize the reaction product (FAST Sigma peroxidase tablet).

A microscopic bioquantification analysis system (Bioquant TCW 98) was used to quantify the amount of immunoreactivity for each of the neurochemicals using the videocount area and field mode options at the selected magnification of x40. A thresholded videocount of the region of interest was used to determine the mean area fraction of immunoreactive product. Three locations within the tendon, epitendon, and paratenon tissues of the forelimb tendons were bioquanted bilaterally. Group means and SEM of area fraction (n = 4/group) were plotted against the week of task performance. Factors week, tendon region, limb, and group were used in a 4-way ANOVA (p ≤ 0.05) to determine differences in SP, CGRP, and NMDAR1 immunoreactivity. Post-hoc analyses were carried out using the Bonferroni method.

RESULTS SECTION:

SP immunoreactivity was significantly increased in peritendon (epitendons-paratenon) at 3 and 12 weeks and endotenon at 3 weeks in HRHF tendons. SP was also present in LRLF 12 week peritendon, but less than HRHF tendons. No SP immunoreactivity was observed in LRLF tendons at 3 or 6 wks, or in control tendons. In contrast, NMDAR1 immunoreactivity was significantly increased only in peritendon and endotenon at 6 weeks in the HRHF tendons. No NMDAR1 immunoreactivity was present above control levels in the LRLF tendons. CGRP immunoreactivity was significantly increased in peritendon at 3 and 12 weeks in HRHF tendons and at 3 and 6 weeks in LRLF tendons. No CGRP immunoreactivity was present above control levels in endotenon. The response was greatest in the HRHF peritendon at week 3 for CGRP, week 6 for NMDAR1 and week 12 for SP. In the LRLF peritendon, the response for CGRP was greatest at week 3 in the nonreach limb.

DISCUSSION:

Our findings demonstrate that SP, CGRP, and NMDAR1 increases in tendon tissues as a consequence of performing highly repetitive and forceful tasks. The response is tissue dependent with a greater response in peritendon than endotenon in the HRHF reach limbs. The presence of increased SP, CGRP, and NMDAR1 in the HRHF nonreach limb is likely due to sustained postural loading during task performance. The tissue response is also dependent upon duration of task exposure. Although the CGRP immunoreactivity was significant in both exposure groups, the response was different for the duration of task exposure. The greatest response was at 3 weeks for both exposure groups. The response in the LRLF tendons decreased at 6 weeks and approached zero by week 12. In contrast, the response in the HRHF tendons decreased at week 6 and was elevated at 12 weeks. The increased CGRP in the LRLF tendons may contribute to the development of degraded reach movement patterns observed in this exposure group.

The increased neurochemical response may be linked to persistent pain associated with tendinopathies of the upper extremity.

REFERENCES:


ACKNOWLEDGEMENTS:

This grant was supported by CDC-NIOSH to MB, American Association of Hand Surgery to JF.