In vivo investigation of ECRB tendons with microdialysis technique—no signs of inflammation but high amounts of glutamate in tennis elbow

Håkan Alfredson[1,3], Björn-Ove Ljung[2], Kim Thorsen[1,3] and Ronny Lorentzon[1,3]

Departments of [1]Surgical and Perioperative Science, Sports Medicine, Umeå University, SE-901 87 Umeå. Tel +46 90 7853951. E-mail: hakan.alfredson@idrott.umu.se; [2]Hand Surgery, Stockholm Söder Hospital, Karolinska Institutet, Stockholm, [3]Musculoskeletal Research, National Institute for Working Life, Umeå, Sweden
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ABSTRACT – We used the microdialysis technique to study concentrations of substances in the extensor carpi radialis brevis (ECRB) tendon in patients with tennis elbow. In 4 patients (mean age 41 years, 3 men) with a long duration of localized pain at the ECRB muscle origin, and in 4 controls (mean age 36 years, 2 men) with no history of elbow pain, a standard microdialysis catheter was inserted into the ECRB tendon under local anesthesia. The local concentrations of the neurotransmitter glutamate and prostaglandin E_2 (PGE_2) were recorded under resting conditions. Samplings were done every 15 minutes during a 2-hour period.

We found higher mean concentrations of glutamate in ECRB tendons from patients with tennis elbow than in tendons from controls (215 vs. 69 μmoL/L, $p < 0.001$). There were no significant differences in the mean concentrations of PGE_2 (74 vs. 86 pg/mL).

In conclusion, in situ microdialysis can be used to study certain metabolic events in the ECRB tendon of the elbow. Our findings indicate involvement of the excitatory neurotransmitter glutamate, but no biochemical signs of inflammation (normal PGE_2 levels) in ECRB tendons from patients with tennis elbow.

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There is general agreement that the extensor carpi radialis brevis (ECRB) muscle plays a central role in the development of tennis elbow (Cyriax 1936, Goldie 1964, Coonrad and Hooper 1973, Regan et al. 1992, Fridén and Lieber 1994, Lieber et al. 1997), but the etiology and pathophysiology of this condition are not clearly understood. Microrupture (Cyriax 1936, Coonrad and Hooper 1973), granulation tissue (Goldie 1964), and degenerative changes without signs of inflammation (Nirschl 1992, Regan et al. 1992) in the ECRB tendon have been reported in histological studies of biopsies from patients with tennis elbow.

Pain is felt in the tendon-part of the muscle (muscle origin), and recently the innervation of the muscle origin in patients with tennis elbow and in controls was investigated. A sensory innervation, restricted to a subpopulation of small blood vessels and nerve fibers showing SP (substance P) and CGRP (calcitonin gene-related peptide)-like immunoreactivity, was foound at the ECRB muscle origin (Ljung et al. 1999a, b).

In situ microdialysis has proved to be a useful technique for study of the metabolism of substances in different types of human tissue (Darimont et al. 1994, Thorsen et al. 1996), and in a recent investigation, we found that this technique can be used to study concentrations of certain substances in the Achilles tendon (Alfredson et al. 1999). It permits continuous measurements of concentrations in vivo of substances with a molecular size below the cut-off limit of the dialysis membrane.

This investigation aimed to find out whether it was technically possible to use microdialysis in the ECRB tendon of the elbow, and to detect and study the local concentrations of glutamate and prostaglandin E_2 (PGE_2) in patients with tennis elbow. Glutamate (excitatory neurotransmitter) and prostaglandin E_2 (involved in inflammatory reactions) have previously been shown to be well suited to examination with microdialysis (Alfredson et al. 1999).

Patients and methods

This study includes 3 male and 1 female patients (mean age 41 (29–54) years) who had had pain for more than 6 months from the ECRB muscle origin and were on the waiting list for surgical treatment of tennis elbow. They all had tenderness over the muscle origin and pain was induced in this region by forced extension of the wrist joint. Differential diagnoses, such as synovitis in the proximal radioulnar joint, entrapment of the radial nerve, compartment syndrome in the anconeus muscle, arthritis and other joint diseases, were excluded by clinical examination.

For comparison, 4 controls (2 women) with a mean age of 36 (28–43) years and no history of a painful elbow condition, were included.

All patients and controls were healthy and not on any medication. The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Umeå, and the experiment was conducted according to the principles expressed in the Declaration of Helsinki.

The microdialysis was performed as a surgical procedure under sterile conditions. With the patient in a supine position, the skin over the lateral part of the elbow and proximal ECRB muscle was disinfected. A local anesthetic (4–5 mL prilocainhydrochloride, 10 mg/mL) was injected into the skin and subcutaneous tissue. In the patient group, a straight, 5–6 cm long skin incision was made over the proximal ECRB muscle. In the control group, we made a straight, 2 cm long skin incision over the proximal ECRB muscle and a 1 cm long incision 4 cm distal to the lateral humeral epicondyle. This was done to minimize the length of the skin incision in the control group. Parts of the fascia over the extensor muscle insertion were incised longitudinally, and the ECRB tendon was identified. The microdialysis catheter (with a diameter of 1.4 mm and length of the membrane covered active part of 30 mm) was introduced into the tendon under visual control, placed longitudinally and parallel to the tendon fibers, and fixed to the skin (Figure 1). Palpation ensured that the distal part of the catheter was placed in the proximal part of the muscle origin. The microdialysis system consists of a battery-driven infusion pump (CMA 106; CMA/Microdialysis AB, Stockholm Sweden) with a fixed infusion rate at 0.3 μL/min. The dialysis catheter (CMA 60; CMA/Microdialysis AB), perfused with Ringer's acetate, has a pore dimension of 20 kD and, at the perfusion rate 0.3μL/min, almost full recovery of PGE_2 is achieved in the dialysate (Darimont et al. 1994). At this rate and membrane dimension, almost full recovery of glutamate can be expected. The void volume from the probe to the sample collector is 3 μL, equivalent to a 10-min fraction. After flushing the system, sampling was done every 15 min, and every dialysis sample (4.5 μL) was immediately frozen to –75 ºC. Samples were taken for up to 2 hours, with the patient resting in a supine position. Samples from two consecutive 15-min periods were pooled.

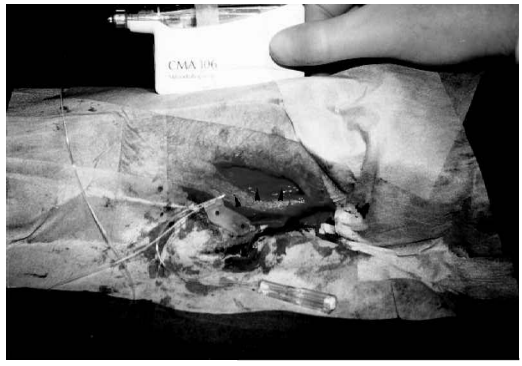

Figure 1. Microdialysis catheter placed in the ECRB tendon (see arrows). Battery-driven infusion pump and sample collector.

After derivatization with OPA 40 mmol/L, glutamate was determined with HPLC (precolumn Nucleosil C18. 5μm, 5 × 4 mm, Knauer, column Nucleosil C18. 5 μm, 60 × 4 mm, Knauer). The mobile phase A contained 0.1 M sodium acetate, pH 6.95, and the mobile phase B methanol: tetrahydrofuran (97.5 : 2.5) and the flow rate was 1.0 mL/minute. Fluorescence detection was done using a CMA/280 Fluorescence Detector (CMA Microdialysis) at excitation 330–365 nm and emission 440–530 nm.

Prostaglandin E_2 was analyzed with a commercially available PGE_2 radioimmunoassay kit (DuPont, Boston, MA). Samples or standards, together with 125I-PGE_2 tracer, were incubated with rabbit anti-PGE_2 antibodies in a 0.0255 M phosphate buffer, pH 6.8, overnight at 4 ºC. The samples were then precipitated by adding poly-

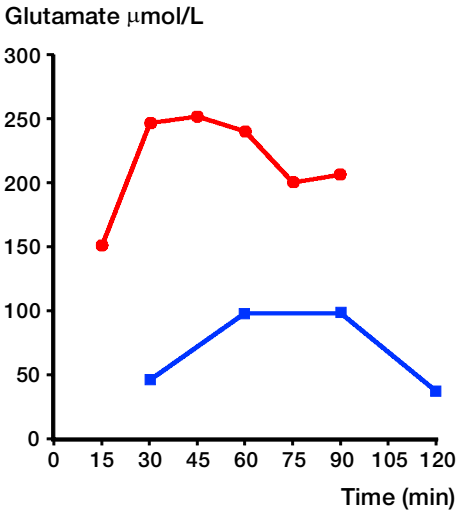

Figure 2. The concentrations (μmol/L) of glutamate (mean) in ECRB tendons (red) with tendinosis (tennis elbow) and in normal ECRB tendons (blue) during the 2-h sampling period. Due to the large variations between the relatively few values, standard deviations are not shown.

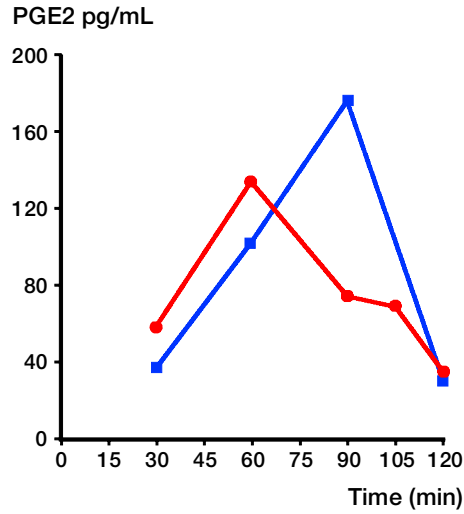

Figure 3. The concentrations (pg/mL) of PGE_2 (mean) in ECRB tendons (red) with tendinosis (tennis elbow) and in normal ECRB tendons (blue) during the 2-h sampling period. Due to the large variations between the relatively few values, standard deviations are not shown.

ethylene glycol, centrifugated, and decanted, and the radioactivity in the pellet was determined by using a gamma counter. The sensitivity of the assay is 10 pg/mL. The antibody used shows a 3.7 % cross-reactivity to PGE1, but < 1% to dehydro-keto-PGE_2, PGA_2, 6-keto-$PGF_{1\alpha}$, PGF_2 and TXB_2.

Surgical procedure

After the microdialysis, additional (4–5 mL) local anesthetic was injected into the skin and subcutaneous tissue, and the patients were operated on. Before the microdialysis catheter was removed from the tendon, careful dissection was done to confirm that the catheter was properly positioned in the tendon. In a modified Hohmann (1933) procedure, the ECRB muscle origin was detached from the bone.

Statistics

An independent-samples test (Mann-Whitney) was used to compare the mean concentrations of glutamate and PGE_2 in the ECRB tendons from the patients with tennis elbow and in the normal ECRB tendons (controls). $P < 0.05$ was considered significant.

Results

The ECRB tendons from patients with tennis elbow had higher mean concentrations of glutamate, compared to the ECRB tendons from the controls (215 (SD 30) vs. 69 (SD 28) μmol/L, $p < 0.001$; Figure 2).

There were no significant differences in the mean concentrations of PGE_2 (74 (SD 24) vs. 86 (SD 53) pg/mL, $p = 0.8$) between the ECRB tendons from the patients with tennis elbow and the tendons from the controls (Figure 3).

Discussion

Our experiment shows that it is possible to use the microdialysis technique to detect and study concentrations of the low molecular substances glutamate and prostaglandin E_2 (PGE_2) in the ECRB tendon of the elbow, and that ECRB tendons in patients with tennis elbow had higher concentrations of the excitatory neurotransmitter glutamate, but not PGE_2, compared to normal ECRB tendons (controls).

Our results are based on relatively few individuals (4 patients and 4 controls). This was mainly due to the painful condition that was seen in the

controls after the microdialysis procedure. The controls had 5–10 degrees of extension deficiency and pain at the extensor origin for 2–3 weeks after the microdialysis procedure. We therefore decided to stop this investigation in controls after studying these 4 subjects.

The microdialysis technique has previously been shown in our clinic to be useful for evaluating bone metabolism in humans by determining the production of PGE_2 in the proximal tibia metaphysis (Thorsen et al. 1996), and studying the local concentrations of glutamate and PGE_2 in the Achilles tendon (Alfredson et al. 1999). When using this method, several difficulties should be taken into account. It is an invasive procedure, and care must be taken to prevent infection. Therefore, an operating room with facilities ensuring sterile conditions when introducing the microdialysis catheter into the tendon is necessary. Moreover, the catheter is soft, the membrane surrounding the catheter is very sensitive, and has to be carefully handled to avoid damage. To prevent such damage, and ensure that it is correctly inserted and placed in the tendon, it should be introduced during palpation under visual control. To prevent clotting in the catheter, the system should be flushed immediately after inserting the catheter in the tendon. The handling of the samples is also important. They should be frozen to –75 °C immediately because of the rapid metabolism of PGE_2.

All patients with tennis elbow had had localized pain at the origin of the ECRB muscle for a long time. To ensure that the catheter was placed in the painful area, we inserted it in the muscle origin under visual control during palpation. After the microdialysis, but before removal of catheter, a careful longitudinal dissection of the ECRB tendon was performed to ensure that it had been placed in the tendon and not in the paratendinous tissue.

We used a local anesthetic without adrenaline to infiltrate the skin and paratenon at the insertion site. This probably does not affect the concentrations of glutamate and PGE_2. However, the tissue trauma caused by insertion of the catheter could primarily affect the concentrations of the neurotransmitter glutamate, but when examining the glutamate concentrations in the different samples over the 2-hour sampling period, there were no signs indicating that this procedure had had any significant effect.

In patients with tennis elbow, although biopsies have shown no inflammatory cells (Nirschl 1992, Regan et al. 1992), many believe that there is an inflammatory reaction. Treatment with anti-inflammatory medication, e.g., NSAIDs and local corticosteroid injections, is often instituted (Kraushaar and Nirschl 1999). Therefore, it was interesting that our in vivo study showed that the concentration of PGE_2, which plays a main role in inflammatory reactions (Solomon et al. 1968), did not differ between the ECRB tendons in patients with tennis elbow and the controls (normal tendons). These results, together with previous results of biopsies (Nirschl 1992, Regan et al. 1992), further indicate that there is no chemical inflammation in the chronic stage of this condition, and treatment should not focus on anti-inflammatory agents. For pain control, agents other than NSAIDs and local corticosteroid injections (with fewer side effects) could be used.

The background and mechanisms of pain in tennis elbow are unknown. However, recent research has clarified the innervation at the ECRB muscle origin, and showed the presence of sensory nerve fibers (Ljung et al. 1999a). Using immunohistochemistry and antibodies to the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP), SP- and CGRP-like immunoreactivity at the muscle origin has been demonstrated (Ljung et al. 1999b). It is known that SP and CGRP have afferent functions (transmitting nociceptive information to the spinal cord), but also efferent functions like involvement in vasodilatation and plasma extravasation (so-called neurogenic inflammation) (Wiesenfeld-Hallin and Xu 1993). Therefore, theoretically, SP and CGRP may somehow be involved in the pain mechanisms associated with tennis elbow.

When using the microdialysis technique, we have as yet no method to measure the concentrations of SP and CGRP, but we do have a method to measure concentrations of the neurotransmitter glutamate (Alfredson et al. 1999). In recent years, the importance of glutamate as a mediator of pain has been emphasized (Dickenson et al. 1997). It is known that glutamate receptors are present in my-

elinated and unmyelinated sensory axons (Coggeshall and Carlton 1998), and that peripherally-administered glutamate antagonists reduce the response to formalin-induced nociception in the rat (Davidson et al. 1997). We found higher concentrations of the excitatory neurotransmitter glutamate in ECRB tendons from patients with tennis elbow than in normal tendons. These findings are in line with those in a microdialysis study in patients with painful chronic Achilles tendinosis, whose tendons had a fourfold higher concentration of glutamate than normal tendons (Alfredson et al. 1999). However, we need to find out whether the higher concentration of glutamate is caused by a local hyperproduction of glutamate or an increase of axonal transport of glutamate into the area with tendon changes (tendinosis). We have preliminary results of biopsies from Achilles tendons showing glutamate receptors (NMDAR1) in relation to nerves.

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