An in vivo microanalytical technique for measuring the local biochemical milieu of human skeletal muscle

Jay P. Shah,[1] Terry M. Phillips,[2] Jerome V. Danoff,[1,3] and Lynn H. Gerber[1]

[1]*Rehabilitation Medicine Department, Clinical Research Center, National Institutes of Health; *[2]*Ultramicro Analytical Immunochemistry Resource, Division of Bioengineering and Physical Science, Office of Research Services, National Institutes of Health, Bethesda, Maryland; and *[3]*Department of Exercise Science, George Washington University, Washington, DC*

Shah, Jay P., Terry M. Phillips, Jerome V. Danoff, and Lynn H. Gerber. An in vivo microanalytical technique for measuring the local biochemical milieu of human skeletal muscle. *J Appl Physiol* 99: 1977–1984, 2005. First published July 21, 2005; doi:10.1152/japplphysiol.00419.2005.—Myofascial pain associated with myofascial trigger points (MTrPs) is a common cause of nonarticular musculoskeletal pain. Although the presence of MTrPs can be determined by soft tissue palpation, little is known about the mechanisms and biochemical milieu associated with persistent muscle pain. A microanalytical system was developed to measure the in vivo biochemical milieu of muscle in near real time at the subnanogram level of concentration. The system includes a microdialysis needle capable of continuously collecting extremely small samples (~0.5 μl) of physiological saline after exposure to the internal tissue milieu across a 105-μm-thick semi-permeable membrane. This membrane is positioned 200 μm from the tip of the needle and permits solutes of <75 kDa to diffuse across it. Three subjects were selected from each of three groups (total 9 subjects): normal (no neck pain, no MTrP); latent (no neck pain, MTrP present); active (neck pain, MTrP present). The microdialysis needle was inserted in a standardized location in the upper trapezius muscle. Due to the extremely small sample size collected by the microdialysis system, an established microanalytical laboratory, employing immunoaffinity capillary electrophoresis and capillary electrochromatography, performed analysis of selected analytes. Concentrations of protons, bradykinin, calcitonin gene-related peptide, substance P, tumor necrosis factor-α, interleukin-1β, serotonin, and norepinephrine were found to be significantly higher in the active group than either of the other two groups ($P < 0.01$). pH was significantly lower in the active group than the other two groups ($P < 0.03$). In conclusion, the described microanalytical technique enables continuous sampling of extremely small quantities of substances directly from soft tissue, with minimal system perturbation and without harmful effects on subjects. The measured levels of analytes can be used to distinguish clinically distinct groups.

myofascial trigger points; musculoskeletal pain; microdialysis; soft tissue pain; pressure pain threshold

MYOFASCIAL TRIGGER POINTS (MTrPs) are a very common cause of clinically observed local muscle pain and tenderness. According to the most commonly accepted theory, the pain associated with MTrPs comes from a hypersensitive nodule in a taut band of skeletal muscle (20).

An "active" MTrP causes pain and may often cause general motor dysfunction (stiffness and restricted range of motion). A "latent" MTrP often causes motor dysfunction without pain. Otherwise, latent MTrPs exhibit all the characteristics of active MTrPs, although usually to a lesser degree. Normal muscle does not contain taut bands or MTrPs (20).

The transformation of a tender nodule into a myofascial pain syndrome is poorly understood. However, local muscle pain is known to be associated with the activation of muscle nociceptors by a variety of endogenous substances including neuropeptides, arachidonic acid derivatives, and inflammatory mediators, among others (11).

Pain is a complex process that involves the interaction of an array of biochemicals, transmitters, and receptors in both the central and peripheral nervous systems. Elucidation of the underlying mechanisms of chronic neuropathic pain is providing clinicians with greater understanding of persistent neuropathic pain and treatment options (12, 24, 25). For example, in vivo and in vitro serological studies of peripheral blood and central nervous system (CNS) assays have shown cytokine tumor necrosis factor-α (TNF-α) to be critically involved in the pathogenesis of neuropathic pain states. In animal models, local TNF-α administration can evoke spontaneous electrophysiological activity in afferent C and A-delta nerve fibers that results in low-grade nociceptive input contributing to central sensitization. Anti-TNF-α agents reduce both the neuropathologic and behavioral manifestations of neuropathic pain states (13).

Nociceptive terminals in muscle display a multitude of different receptor molecules in their membranes, including matched receptors for well-documented endogenous substances such as bradykinin, 5-hydroxytryptamine (5-HT or serotonin), protons (H^+), and prostaglandins that are released from damaged tissue. These biochemicals will bind with their matched receptors on the nociceptors bringing the membrane closer to threshold for an action potential. When summation is sufficient, action potentials will result, leading to local muscle pain and tenderness (11). Furthermore, as bradykinin, 5-HT, and H^+ sensitize the muscle nociceptors (i.e., lower than their normally high stimulation threshold), the sensitized muscle nociceptors are then more easily activated and may respond to normally innocuous and weak stimuli such as light pressure and muscle movement.

The continued presence of such biochemicals (and others) as discussed above may be a necessary condition for persistent pain. However, little is known about the biochemical differences in the local tissue milieu between normal muscle and muscle with painful or nonpainful MTrPs, especially with respect to muscle contraction. Yet the pathophysiology, physical findings, and treatment methods of myofascial pain involve the local soft tissue (11, 20).
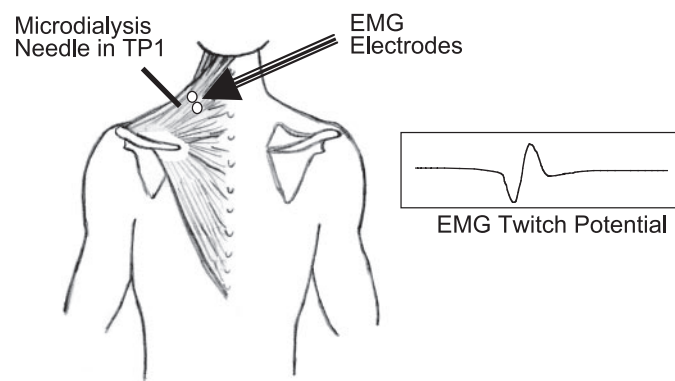
Fig. 1. Needle location in upper trapezius. TP1, trigger point 1 (TP1). EMG potential during local twitch response.

Eliciting the local twitch response (LTR)[1] via "dry needling" of MTrPs often produces a therapeutic benefit (Fig. 1) (20). Local diagnostic methods such as thermography, EMG, biopsy, etc., have not elucidated the pathophysiology or shown changes after treatment. Therefore, assaying the local milieu before, during, and after the LTR could potentially describe changes in bioactive substances that may contribute to persistent pain and to the effect of local treatment. This could then lead to the development of treatments targeted at those underlying mechanisms and to a better understanding of the relationship between these substances and the neuroplastic changes in the CNS that occur in chronic myofascial pain.

In this communication, we describe the use of a novel microdialysis needle (designed, fabricated, and tested by the investigators) combined with specialized microanalytical techniques pioneered by one of the investigators (T. M. Phillips; 15–17).

This study was undertaken to *1*) determine if this device and these techniques can successfully sample the in vivo biochemical milieu of muscle and identify changes associated with a rapid muscle twitch in near real time at the subnanogram level of concentration and *2*) demonstrate proof of principle of the technique's ability to distinguish among subjects who have clinically distinct soft tissue findings.

METHODS

Instrumentation

Microanalytical system. In vivo microdialysis is used to measure the chemical composition of interstitial fluid by means of a semipermeable membrane at the tip of a probe. The inner surface of the probe membrane is constantly perfused with physiological saline, and when the probe is implanted into tissue, molecules present in the interstitium diffuse across the membrane (down their concentration gradient) into the perfusion medium of the probe. Microdialysis enables in vivo sampling and measurement of tissue chemistry, and this technique has been applied to studies of human muscle, blood, adipose tissue, ocular tissues, brain, and liver. Its use is feasible in virtually every human organ (2).

A prototype needle microdialysis system was developed comprising a hollow small-bore needle equipped with a microdialysis mem-
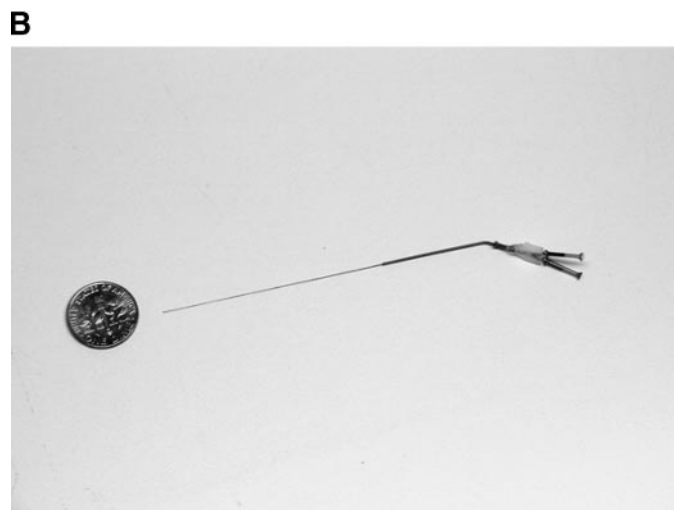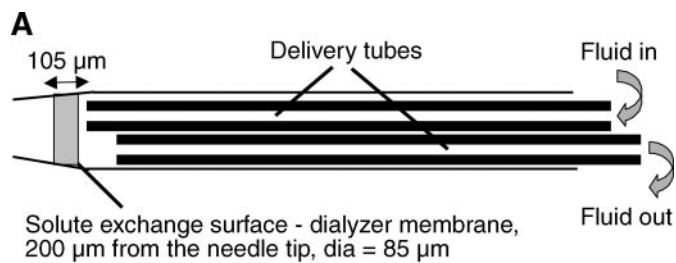
brane, standard microdialysis connection tubing, a microdialysis perfusion pump, and a sample collection device. The system was designed to continuously collect samples from the internal tissue milieu of human muscle and could be used as a surrogate acupuncture needle during routine treatment of MTrPs (Figs. 2, *A* and *B*, and 3).

Needle construction. One of the investigators (T. M. Phillips) designed and fabricated the needle in the Bioengineering and Physical Science Division of the Office of Research Services, National Institutes of Health. The needle was constructed from a 2.5-in. section of 30-gauge commercially available stainless steel hypodermic tubing (Small Parts, Miami Lakes, FL). One end was carefully ground internally and externally before being polished to a cone to minimize tissue injury in a similar manner to that of a standard Japanese-style acupuncture needle.

An 85-μm-diameter disk was cut from a 105-μm-thick sheet of cellulose ester semi-permeable membrane (Spectrum Laboratories,

Fig. 2. *A*: schematic of microdialysis needle construction. *B*: microdialysis needle.
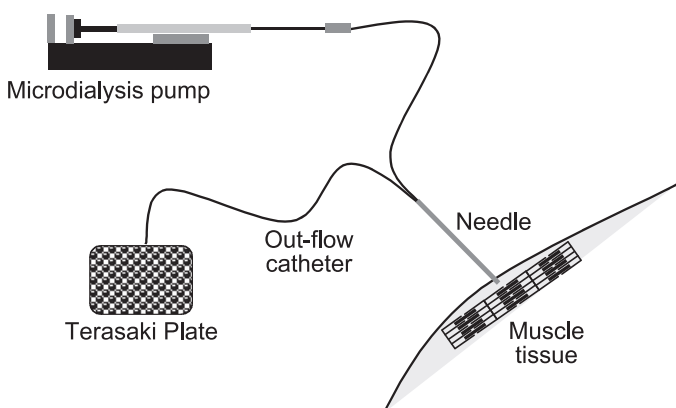

Fig. 3. Schematic of perfusion pump and collection plate.

---

[1] Local twitch response refers to the brief, local contraction that occurs when a trigger point is stimulated by snapping palpation or needle penetration. Dry needling refers to stimulation of a trigger point, usually with a narrow object such as a blunt needle or empty hypodermic needle.

Rancho Dominguez, CA) and placed on a specially designed jig. The jig enabled the precise positioning and cementing into place of the membrane exactly 200 μm from the ground tip of the needle.

Two extruded polyethylene tubes (Small Parts) with the ends precisely offset by 100 μm from each other were glued together and positioned 200 μm from the membrane (Fig. 2, *A* and *B*). The dual-tube assembly was sealed into place and the proximal ends of the tubes were attached to 1-m length standard fluoropolymer FEP microdialysis tubing via expandable tubing adaptors (0.12-mm-ID tubing; CMA Microdialysis, North Chelmsford, MA). The inlet tubing was attached to a model 102 microdialysis pump equipped with a 2.5-ml glass infusion syringe (CMA Microdialysis), whereas the outlet tubing went to the sample collection device.

The syringe was loaded with 2.0 ml of sterile saline, pH of 7.0, which was used to prime or flush the microdialysis needle. Collection of the dialysate was manually achieved by placing the end of the outlet tubing into a 20-μl well of a 72-well Terasaki microculture plate (Robbins Scientific, Sunnydale, CA; Fig. 3). Consecutive samples were collected at predetermined time intervals by moving the catheter outlet from one well to another in an orderly manner. Each sample was collected under mineral oil to prevent loss by evaporation and to facilitate sample recovery and handling.

Characterization and calibration of the microdialysis system. The flow efficiency of the needle was tested by injecting a dilute dye through the needle and checking that the time taken for the dye to traverse the complete system was comparable with that calculated theoretically. Considering the length (∼1 m) of the FEP tubing at the inlet and outlet, it seemed reasonable to check flow and pressure. These were found to be within acceptable limits, and the needle was then subjected to further testing and calibration. The characteristics and efficiency of the microdialysis system were tested by placing the needle in a solution of saline containing commercially available molecular mass standards ranging from 1 to 150 kDa (Sigma-Aldrich, St. Louis, MO). The system was also tested for recovery and precision by repeatedly sampling the molecular mass standard solution and analyzing recoveries. Additionally, standard solutions of the 10 different analytes of interest were prepared in either saline or a soluble saline extract of postmortem (∼2 h postdeath with family consent) human muscle. The muscle extract solution was used to test the effects of muscle tissue fluid on analyte recovery. A mixture of equal parts of each analyte was prepared, and the microdialysis needle was used to sample the mixtures at flow rates of 1 μl/min and 2 μl/min with samples collected at 0.5 and 0.25 min, respectively. Our Institutional Review Board did not approve in vivo calibration; therefore, the same mixture was deposited in defined areas of an isolated, perfused (rat trapezius) muscle. The needle was precisely inserted into these areas via a stereotactic positioner (Harvard Apparatus, Holliston, MA), and the deposits were sampled at identical rates as described for the fluid samples. All recoveries were performed in triplicate.

Sample analysis. Due to the extremely small volumes collected from the microdialysis system (∼0.5 μl), analysis of each sample was performed in the Ultramicro Analytical Immunochemistry Resource (UAIR) by immunoaffinity capillary electrophoresis (ICE) and capillary electrochromatography (CEC). Measurement pH was made with a modified microcombination electrode in combination with an Orion model 370 pH meter (Thermo Electron, Woburn, MA) capable of making pH measurements in ∼0.2 μl of fluid. Samples were examined within 4 h of collection, and pH measurements were made immediately on arrival in the UAIR. Each sample was recovered from the Terasaki plate, measured for volume, and stored at −80°C until analyte analysis by ICE and CEC. The major advantages of ICE and CEC are the extremely small sample volumes required for analysis (∼50 nl or 0.05 μl) and their high detection sensitivities (∼0.5 pg/ml). An added advantage of ICE is that the employment of an antibody-based initial extraction, followed by laser-induced fluorescence detection allows specific analytes to be isolated and measured in complex

biological fluids; for example, the interstitial fluids surrounding the muscle trigger points.

ICE analysis of microdialysis samples. Measurement of the inflammatory cytokines (IL-1β, TNF-α), pain-associated neuropeptides [calcitonin gene-related peptide (CGRP), substance P (SP)], and bradykinin was performed as previously described (15–17). Briefly, antibody fragments (FAb) were prepared from antibodies specific to the analyte and immobilized onto 4 cm of the inner surfaces of a 100-μm ID fused silica capillary (Polymicro Technologies, Phoenix, AZ) via a disulfide linkage (15–17). The antibody-coated capillary was mounted into a Crystal 660 Capillary Electrophoresis system (Prince Technologies, Amsterdam, Netherlands) equipped with a Zetalif laser-induced fluorescence detector and a 12 mW 633 helium-neon laser (Picometrics, Ramonville, France). A 50-nl volume of collected sample was vacuum injected into the capillary and allowed to incubate with the immobilized antibodies for 5 min, during which each of the different antibodies interacted with and bound their specific analyte. Nonbound materials were flushed out of the capillary and collected for further analysis. The bound analytes were labeled in situ with Alexa-Fluor 633 laser dye (Molecular Probes, Eugene, OR), flushed to remove nonreactive material, and the bound analytes were then electroeluted at pH 1.5. The individual analytes were separated by electrophoresis at 175 μA constant current, and the individual peaks were analyzed by the fluorescence detector using DAX peak area analytic program (Prince Technologies). All peak areas were then compared with those obtained by analyzing standard curves of each analyte.

CEC analysis of microdialysis samples. Measurement of serotonin and norepinephrine was performed on a Micro-Tech Scientific Ultra-Plus II MD CEC system (Micro-Tech Scientific, Vista, CA) equipped with a 100 mm × 100 μm ID capillary column packed with 5 μm C$_{18}$ particles. A 20-nl volume of collected sample was injected by electrical migration into the system and processed at a constant voltage of 15 kV for 30 min according to a modification of the technique described by Oguri et al. (14). Detection of the individual analytes was achieved by filling the column with the amine coupling agent, *o*-phthalaldehyde-2-mercaptoethanol dissolved in borate buffer, pH 10, and allowing each separated analyte to react, via their free amino groups, with the *o*-phthalaldehyde to form fluorescent-labeled compounds. These products were detected online at 340 nm using the system's fluorescence detector. Areas under the peaks were analyzed and compared with known standards run under identical conditions.

Results/characterization and calibration of the microdialysis system. Recovery of the molecular mass standards demonstrated that the largest standard to be reliably recovered was 75,000 Da, with maximum recovery efficiency (<98%) in the molecular mass range of 0.5–45 kDa. The membrane was capable of being regenerated ∼50 times before recovery efficiency dropped below 90% and the system failed to recover the 75 kDa standard. Recoveries of the analytes of interest from both saline and human muscle extract at the two different flow rates are shown in Table 1 along with the recovery of the rat muscle-deposited analyte mixture. Although recoveries were acceptable for all analytes when sampled at 1 μl/min, recoveries at 2 μl/min demonstrated higher recoveries of all analytes, recoveries being >86% in both the fluid samples and the tissue deposits. Collected samples were examined for volume variation and the presence of air bubbles. The mean volume collected per patient was 0.5 ± 0.003 μl and the presence of air bubbles was undetectable in any of the patient samples.

Both ICE and CEC are established analytic techniques known to yield reliable results. The antibodies used in the ICE technique were carefully selected based on no cross-reactivity with the other analytes of interest when tested by two-dimensional Western blotting and mass spectrometry. Absolute identification of the captured or isolated analytes was confirmed by matrix-assisted laser desorption time-of-flight mass spectrometry. The precision and accuracy of the analytical system was measured by running a standard sample five times on the

Table 1. *Recovery of analyte standards from saline, human muscle extract, and isolated rat trapezius muscle in-situ deposition*

Analyte	Medium	Percentage Recovery at Different Flow Rates	
		1 μl/min	2 μl/min
Bradykinin	Saline	85.6±6.2	94.6±3.6
	Tissue extract	86.6±8.8	93.8±5.4
	In situ	88.4±7.2	95.0±5.8
CGRP	Saline	95.5±3.8	97.6±2.1
	Tissue extract	94.9±4.2	96.8±3.5
	In situ	93.7±5.5	96.2±3.2
SP	Saline	95.6±4.2	98.2±1.2
	Tissue extract	94.9±4.1	97.2±2.2
	In situ	92.1±5.6	96.3±2.8
TNF-α	Saline	96.9±2.8	98.1±1.1
	Tissue extract	94.6±3.9	96.3±3.4
	In situ	92.5±5.1	93.4±5.3
IL-1β	Saline	97.8±0.8	98.1±0.6
	Tissue extract	96.9±1.3	97.2±1.4
	In situ	95.5±1.8	96.7±2.9
Serotonin	Saline	89.7±9.3	92.0±8.3
	Tissue extract	83 ±16.3	89.3±10.3
	In situ	84.7±9.8	90.5±9.0
Norepinephrine	Saline	92.3±8.0	96.0±3.8
	Tissue extract	84.0±13.5	92.3±7.0
	In situ	86.5±10.3	89.8±8.8

Values are means ± SD. Analyte standards are a standard mixture containing 40 nM serotonin and norepinephrine; 50 pM bradykinin, and 100 pg of substance P (SP) calcitonin gene-related peptide (CGRP), and the 4 cytokines at pH 7.4.

same day and on 5 consecutive days. The intra-assay coefficients of variance (CV) for the panel of analytes was found to be <5.88 ± 0.21, and the interassay CV was <6.04 ± 0.31. Additionally, recoveries of analytes from standard solutions were shown to be >97.4% for all analytes. On the basis of these findings, we believed that these techniques would then be usable to distinguish the clinically distinct groups identified for this study.

Subjects

Eligible subjects were recruited from among clinical center staff. To be eligible, they completed a Brief Pain Inventory (BPI) and underwent a standard musculoskeletal examination by a single examiner, including palpation for MTrPs at trigger point 1 (TP1) (Fig. 1; Ref. 20). This examiner (J. P. Shah) is a practicing physician with 12 years experience in the evaluation and treatment of musculoskeletal disorders and an instructor in the Harvard Continuing Medical Education Structural Acupuncture for Physicians program. Subjects were assigned to one of three groups based on history and physical examination: *group 1)* normal (no neck pain, no MTrP); *group 2)* latent (no neck pain, latent MTrP present); *group 3)* active (continuous idiopathic cervical pain of <3 mo duration, MTrP present). The first three subjects to qualify for each group were used, yielding a total of nine subjects. Additional qualifiers were identified for later use in data gathering procedures.

Exclusion criteria for all groups included fibromyalgia, cervical radiculopathy, infection, cancer, history of local treatments or medications, history of smoking, and psychological issues such as fear of needles.

This protocol was approved by an Institutional Review Board at the National Institutes of Health, and all subjects signed an Institutional Review Board-approved informed consent.

Procedures

A pressure algometer (Pain Diagnostics and Treatment, Great Neck, NY) was used to measure the local tenderness [pressure pain threshold (PPT)]. Pressure algometry was performed bilaterally on all subjects at TP1. Algometry procedures had been previously determined to be valid and reliable (4).

The subject was comfortably positioned prone on a standard clinical plinth. Two pillows were used to support and stabilize the subject. Then the microdialysis needle was inserted into the upper trapezius muscle without penetrating the MTrP (if present). The needle remained in situ for 1 min before sample collection began. Dialysate was sampled according to the following schedule: every minute for the first 4 min, then every 10 s for 1 min (*minute 4 to 5*). At 5 min after needle insertion, the needle was advanced ~1.5 cm deeper into the muscle until a LTR was obtained in *groups 2* (latent) and *3* (active); a LTR was not observed in *group 1* (normal). Recovery of dialysate continued every 10 s for the next 4 min and then every minute for 5 min. Total collection time was 14 min (4 + 1 + 4 + 5). Needle flow rate was maintained at 1 μl/min for the first 4 min and the final 5 min. For the middle 5 min (when samples were collected every 10 s), the flow rate was 2 μl/min.

To confirm the presence or absence of a LTR, simultaneous surface electromyography was performed using a Nicolet EMG Unit (Nicolet Instrument Technologies, Madison, WI; Fig. 1).

Each sample (39 per patient) was analyzed by ICE, CCE, and micro-pH for the following analytes: hydrogen ion (indication of pH), bradykinin, CGRP, SP, TNF-α, IL-1β, serotonin, and norepinephrine.

Three microdialysis needles were fabricated for this study. They were gas-sterilized between uses.

Statistical Design and Analysis

Data for each of the three groups (normal, latent MTrP, active MTrP) were averaged and plotted across time. On the basis of clinical experiences of the principal investigator with patients similar to these subjects, no demonstrable differences were anticipated between males and females and across differing ages. Therefore, this study did not attempt to distinguish results between sexes and across ages.

Algometer pressure responses (PPT) were compared across the three groups by using a simple one-way ANOVA to determine if any of the three groups differed in tenderness from the others.

Two-way repeated ANOVA with "time" as the repeated variable and "group" as a nonrepeated variable was used to compare levels of each analyte. The time variable was limited to three levels (pre-, peak, and post-needle advancement). "Pre" refers to analyte level found 2 min after needle insertion, but before muscle twitch. For the normal and latent groups, the values at 2 min were nearly identical to the values at 1, 3, and 4 min. For the active group, the analyte values at 2 min were very close to the values at 1 min. Therefore, 2 min was selected to represent the pre level. "Peak" refers to the relatively peak metabolite value determined after the twitch (or after the needle advancement for the normal group). For consistency of the data set we chose 5 min after insertion to represent the peak level. "Post" refers to recovery values measured 11 min after needle insertion, which was 6 min after the needle movement.

Data variability is represented by the standard error of the estimate (SEE), which represents the dispersion of a data set around a line of regression. This is analogous to a standard deviation (SD), except that a SD varies around a single mean, whereas a SEE varies around a curve (i.e., regression line). The SEE designates an envelope around the regression line with ~70% certainty. It is based on all points in the curve and is the same throughout the curve. For clarity in the figures, the SEE is indicated around only one point per curve, but this "error window" exists around every data point on the curve.

Table 2. *Significant analyte differences*

Analyte	3 Times Combined: Pre (2 min), Peak (5 min), Post (11 min)	Peak (5 min)
pH (inv H$^+$ concentration)	Act<latent, normal ($P < 0.03$)*	
Bradykinin	Act>latent, normal ($P < 0.01$)	
CGRP	Act>latent, normal ($P < 0.01$)	Act>latent>normal ($P < 0.02$)
SP	Act>latent, normal ($P < 0.01$)	Act>latent>normal ($P < 0.02$)
TNF-α	Act>latent, normal ($P < 0.001$)	
IL-1β	Act>latent, normal ($P < 0.001$)	
Serotonin	Act>latent, normal ($P < 0.01$)	
Norepinephrine	Act>latent, normal ($P < 0.01$)	
Group, Analyte	Significance	
Active, CGRP	Post<Pre, Peak ($P < 0.02$)	
Active, SP	Post<Pre, Peak ($P < 0.02$)	

Act, active. *Significance level.

RESULTS

The active group had a lower PPT ($P < 0.08$), which, although not a low enough probability to achieve classical significance (0.05), might suggest greater tenderness or sensitivity. Overall (i.e., for all 3 time levels combined, 2, 5, and 11 min after needle insertion), the amounts of bradykinin, CGRP, SP, TNF-α, IL-1β, serotonin, and norepinephrine were significantly higher in the active group than the other two groups ($P < 0.01$ or better). Overall, pH was significantly lower in the active MTrP group than the other two groups ($P < 0.03$). At peak time (5 min after the start of data collection when the needle was advanced and the latent MTrP and active MTrP groups demonstrated twitches), peak values of CGRP and SP were significantly different in all three groups (active>latent> normal, $P < 0.02$). In the active MTrP group, the post (11 min after needle insertion and 6 min after needle advancement) values of SP and CGRP were significantly lower than the pre (2 min after needle insertion) and peak values ($P < 0.02$). Specific statistical differences are summarized in Table 2. Only significant comparisons are shown. Graphs of the analytes averaged over all subjects and including all data points can be seen in Figs. 4–7. Times used for the analysis (pre, peak, post) are indicated by vertical arrows, and the SEE (based on all data points) are indicated by vertical bars.

DISCUSSION

Recovery of the sample analytes is dependent on their concentrations in the local milieu and the physical parameters under which they are collected. Flow rate of the perfusate may have a major influence on the total collected solutes because diffusion is a rate-dependent property of the membrane-solution interface. If the flow rate is too rapid, insufficient contact time will be available for representative diffusion to take place. If the flow rate is too slow, gradients across the membrane may

decrease in any local sample sufficiently to reduce diffusion. Previous work has demonstrated that flow rates in the range of 1 to 2 μl/min will allow a proportioned diffusion to take place, which can be used to calculate percentages of solutes in the local tissue milieu (1, 3, 5, 8, 18).

Changes in flow rate are likely to account for some of the apparent increase in all analyte levels between 4 and 5 min. However, the increases up to the peak at 5 min did not achieve significance when compared with the initial levels as represented by data at the 2-min collection time. Another general observation is that for the active group, concentrations for all physical analytes appear to increase (although not statistically significantly) during the initial 4-min period when the needle was in the muscle. During this time the needle was close to, but not within, the MTrP. For the latent and control groups, the initial 4-min baselines were extremely stable. This may be indicative of a greater sensitivity to mechanical stimuli for the active group compared with the other groups, and this sensitivity may extend beyond the MTrP to relatively normal
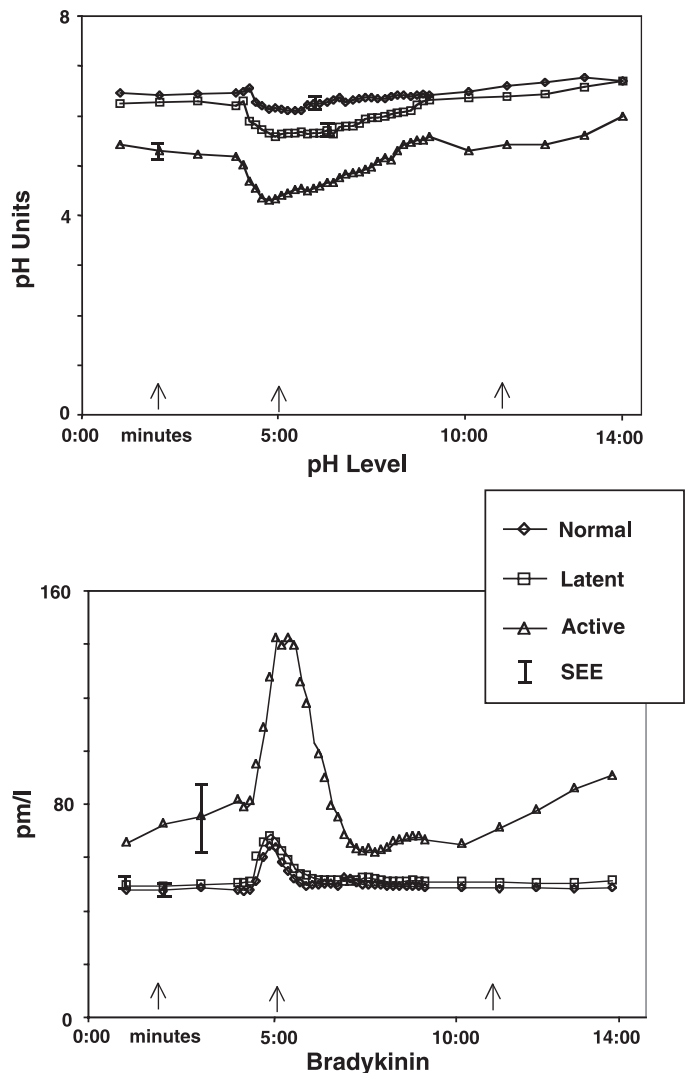

Fig. 4. pH level and concentration of bradykinin over time. For Figs. 4–7, flow rates were 1 μl/min from start until 4 min, 2 μl/min from 4 until 9 min, and 1 μl/min from 9 until 14 min. Vertical arrows indicate times used for statistical analysis (2, 5, and 11 min).
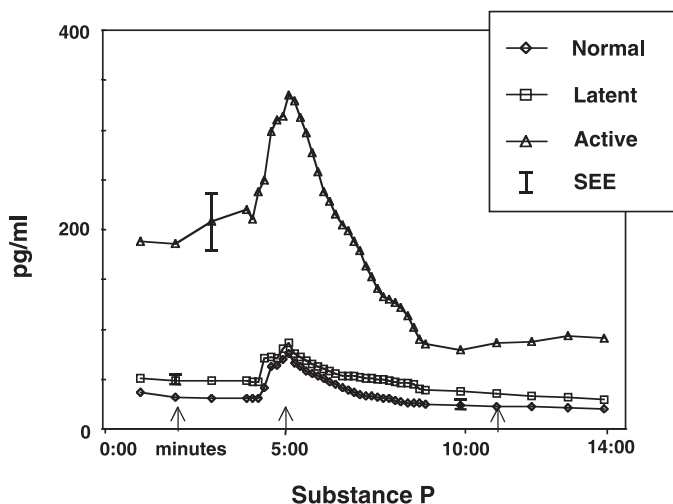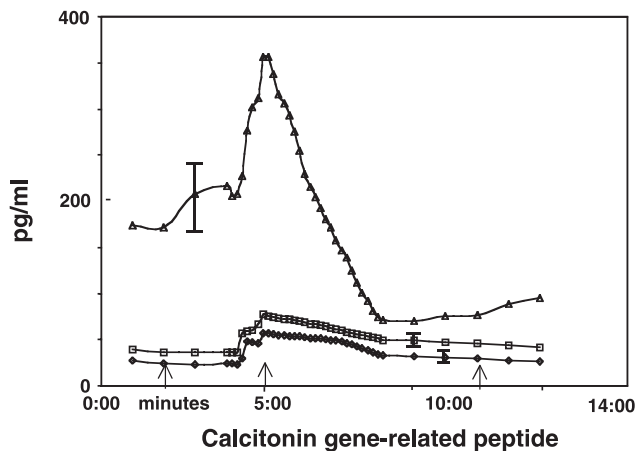
Fig. 5. Concentrations of calcitonin gene-related peptide (CGRP) and substance P (SP) over time.

long collection time intervals would be equivalent to smoothing a curve, which would tend to mask rapid changes in the measured analyte. We hoped to be able to track changes more rapidly in analyte levels by using samples from relatively short collection intervals, which may offer insight into control mechanisms or temporal sequence of events. The ability to distinguish our different subject populations with short collection intervals appears to be supported by the results. Rapid changes in analyte levels may possibly be identifiable; however, additional data with larger samples will be necessary to statistically support this.

The lower PPT found in subjects in the active group supports the well-known observation that people with active MTrPs are more sensitive to external mechanical stimuli. This may be due to general shifts in the nociceptor membrane potentials closer to action potential threshold, or it may be due to the presence of noxious metabolites that bombard and sensitize the membrane receptors. A combination of these two effects is also possible. However, in the current study, these PPT changes have only been observed locally, i.e., directly over the MTrP. Any changes in membrane sensitivities cannot be assumed to be generalized.

Differences in analyte concentration between the active group and the other two groups are not necessarily due to the MTrP. In fact, most differences were apparent from the time of needle insertion. These may be due to increased sensitivity of

Fig. 6. Concentrations of tumor necrosis factor (TNF)-α and interleukin (IL)-1β over time.

muscle tissue. We cannot claim that these are absolute levels of concentration. The important statistical finding is that for almost all comparisons, analyte levels of the active group are higher than the other groups (pH is lower).

Shortly after movement of the needle (5 min), analyte levels dropped. For the active group, we believe this is associated with chemical changes accompanying the muscle twitch. Why the other two groups also show decreases is not clear, but may be due to some similar local response associated with movement of the needle. All interpretations should be applied only to local conditions. Ongoing data collection in our clinic is being performed with strictly constant perfusate flow velocities to allow unbiased study of the analyte concentration shifts surrounding the needle movement. Data are also being collected from a remote, unaffected muscle site for comparison.

Previous studies using microanalytical needle techniques have used collection intervals ranging from 30 min to as much as several hours (10). This is done to assure sufficient equilibration time for the concentrations in the perfusate to match the levels of interstitial analytes. Because equilibration is a time-based function, we postulated that even if equilibration were not complete, proportionate relationships among different subject populations would still be identifiable. In addition, using

Fig. 7. Concentrations of serotonin and norepinephrine over time.

previously been shown between pain and local acidity (7). In a rat model, repeated injections of acidic saline into one gastrocnemius muscle produced bilateral, long-lasting mechanical hypersensitivity (i.e., hyperalgesia) of the paw (22). The hyperalgesia was reversed by spinally administered μ- or ∂-opioid receptor agonists (23) or N-methyl-D-aspartate (NMDA) or non-NMDA ionotropic glutamate receptor antagonists (21). This model clearly demonstrates secondary mechanical hyperalgesia that is maintained by neuroplastic changes in the CNS. Furthermore, the persistent mechanical hyperalgesia was not caused by muscle tissue damage and was not maintained by continued nociceptive input from the site of injury (22). Therefore, an acidic milieu alone (without muscle damage) is sufficient to cause profound changes in the properties of nociceptors, axons, and dorsal horn neurons (i.e., the pain matrix). Mechanical hyperalgesia is a hallmark of a MTrP. An acidic pH is well known to stimulate the production of bradykinin during local ischemia and inflammation and may explain the cause of pain in patients with an active MTrP.

We found significantly elevated levels of SP and CGRP in the vicinity of the active MTrPs (Fig. 5). SP and CGRP are produced in the dorsal root ganglion and over 90% of these biochemicals are transported antidromically down the neural process. There is a constant basal release of small amounts of these substances from the nociceptor into its local milieu (25). However, their release is greatly increased in response to nociceptor activation (e.g., by H^+ and bradykinin binding to their matched receptors). Small amounts of SP are also transported orthodromically from the dorsal root ganglion into the dorsal horn of the spinal cord. Prolonged nociceptor activation is known to greatly increase this process and directly lead to neuroplastic changes in the dorsal horn. This causes profound changes in neuronal activity and the perception of pain.

Both SP and CGRP dropped significantly after the muscle twitch. This concurs with the commonly observed (at least temporary) decrease in pain after the "release" of a MTrP. Physiologically, this may be due to interference with nociceptor membrane channels or transport mechanisms associated with a briefly augmented inflammatory response. Levels of these analytes may also fall due to a local increase in blood flow. Additional study would be appropriate on these analytes.

We found significantly elevated levels of TNF-α and IL-1β in subjects with active MTrPs (Fig. 6). In a rat model, TNF-α produces a time- and dose-dependent muscle hyperalgesia within several hours after injection into the gastrocnemius or biceps brachii. This hyperalgesia was completely reversed by systemic treatment with the nonopioid analgesic metamizol (19). Furthermore, TNF-α did not cause histopathological tissue damage or motor dysfunction. One day after injection of TNF-α, elevated levels of CGRP, nerve growth factor (NGF), and PGE$_2$ were found in the muscle. Therefore, TNF-α and other proinflammatory cytokines such as IL-1β may play a role in the development of muscle hyperalgesia, and the targeting of pro-inflammatory cytokines might be beneficial for the treatment of muscle pain syndromes (19).

SP causes mast cell degranulation with the release of serotonin (in addition to histamine) and upregulation of proinflammatory cytokines. Increases in TNF-α stimulate the production of norepinephrine. We found significantly elevated levels of serotonin and norepinephrine in subjects with active MTrPs (Fig. 7). The increased levels of norepinephrine may be asso-

the active group to external stimulation (the needle occupying volume within the tissue). Although the microdialysis needle has been made as small as currently is feasible, we cannot rule out the possibility that the very act of data collection is influencing the system. Additional procedures are being developed to isolate the active response from both local and remote tissue milieus. Some local changes are also to be expected with variations in blood flow, in particular bradykinin (9). The active group might have different blood flow properties, and changes in blood flow (which were not recorded in this study) might alter membrane recovery properties or interstitial concentrations. Future studies to compare blood flow properties would contribute to better understanding of this phenomenon.

Several comparisons of analyte levels at the times representing pre, peak, and post levels appear to be different in the figures for the individual groups. However, statistically, these were not significant, and we believe that this lack of significance is due to inadequate power. Overall testing (3 times combined) achieved significance because the n (sample size) becomes effectively larger for combined data. Future studies are planned to investigate the changes in analytes for specific groups.

In our study, subjects with active MTrPs and greater pain levels (i.e., pressure sensitivities) had lower pH levels in the vicinity of their MTrPs (Fig. 4). A positive correlation has

ciated with increased sympathetic activity in the motor end plate region, which could then account for the lower threshold of a LTR.

The observations from our study support the assertion that a minimally invasive local sampling tool can safely gather a large amount of data related to myofascial pain, yet be sensitive enough to identify changes across conditions and clinical states.

In conclusion, our microdialysis system, using samples of <1 μl, is capable of continuous, near real time, in vivo recovery of molecules 75 kDa and smaller directly from the soft tissue environment without harmful effects on subjects. Subsequent analysis of the collected samples can distinguish analyte levels before, during, and after a LTR. In this preliminary proof of principle investigation, differences have been demonstrated in the level of these analytes between people who have pain and those who do not and between those who have active MTrPs versus those who have latent or no MTrPs. The local milieu does appear to change with the occurrence of the LTR, and these changes can be tracked with short (minutes) collection intervals.

Exploration of the biochemical milieu of MTrPs and normal muscle may help explain the pathogenesis, persistence, and amplification of myofascial pain. Treatment rationales and techniques may also evolve from this information. Dry needling, for example, has already been shown to have some clinical efficacy (6). In this report, techniques are described that have the potential to identify and quantify biochemical changes associated with therapeutic interventions.

REFERENCES

1. **Brown SA, Mayberry AJ, Mathy JA, Phillips TM, Klitzman B, and Levin LS.** The effect of muscle flap transposition on TNF-α levels during fracture healing. *Plast Reconstr Surg* 105: 991–998, 2000.
2. **Chaurasia CS.** In vivo microdialysis sampling: theory and applications. *Biomed Chromatogr* 13: 317–332, 1999.
3. **Connelly CA.** Microdialysis update: optimizing the advantages. *J Physiol* 514: 567–578, 1999.
4. **Fischer AA.** Algometry in diagnosis of musculoskeletal pain and evaluation of treatment outcome: an update. *J Musculoskeletal Pain* 6: 5–32, 1998.
5. **Hamin K, Rosdahl H, Ungerstedt U, and Henriksson J.** Microdialysis in human skeletal muscle: effects of adding a colloid to the perfusate. *J Appl Physiol* 92: 385–393, 2002.
6. **Hong CZ.** Lidocaine injection versus dry needling to myofascial trigger point. The importance of the local twitch response. *Am J Phys Med Rehabil* 73: 256–263, 1994.
7. **Issberner U, Reeh PW, and Steen KH.** Pain due to tissue acidosis: a mechanism for inflammatory and ischemic myalgia? *Neurosci Lett* 208: 191–194, 1996.
8. **Kehr J.** A survey on quantitative microdialysis: theoretical models and practical implications. *J Neurosci Methods* 48: 251–261, 1993.
9. **Langberg H, Bjorn C, Boushel R, Hellsten Y, and Kjaer M.** Exercise-induced increase in interstitial bradykinin and adenosine concentrations in skeletal muscle and peritendinous tissue in humans. *J Physiol* 542: 977–983, 2002.
10. **Lott MEJ and Sinoway LI.** What has microdialysis shown us about the metabolic milieu within exercising skeletal muscle? *Exerc Sport Sci Rev* 32: 69–74, 2004.
11. **Mense S and Simons DG.** *Muscle Pain: Understanding Its Nature, Diagnosis and Treatment.* Baltimore, MD: Lippincott, Williams and Wilkins, 2001.
12. **Moore KA, Baba H, and Woolf CJ.** Gabapentin—actions on adult superficial dorsal horn neurons. *Neuropharmacology* 43: 1077–1081, 2002.
13. **Myers RR, Wagner R, and Sorkin LS.** Hyperalgesia actions of cytokines on the peripheral nervous system. In: *Cytokines and Pain: Progress in Inflammation Research,* edited by Watkins L. Basel: Birkhauser Verlag, 1999, p.133–158.
14. **Oguri S, Yoneya Y, Mizunuma M, Fujiki Y, Otsuka K, and Terabe S.** Selective detection of biogenic amines using capillary electrochromatography with an on-column derivatization technique. *Anal Chem* 74: 3463–3469, 2002.
15. **Phillips TM and Dickens BF.** Analysis of recombinant cytokines in human body fluids by immunoaffinity capillary electrophoresis. *Electrophoresis* 19: 2991–2996, 1998.
16. **Phillips TM.** Analysis of single-cell cultures by immunoaffinity capillary electrophoresis with laser-induced fluorescence detection. *Luminescence* 16: 145–152, 2001.
17. **Phillips TM.** Determination of in situ tissue neuropeptides by capillary immunoelectrophoresis. *Anal Chim Acta* 372: 209–218, 1998.
18. **Rosdahl H, Hamrin K, Ungerstedt U, and Henriksson J.** A microdialysis method for the in situ investigation of the action of large peptide molecules in human skeletal muscle: detection of local metabolic effects of insulin. *Int J Biol Macromol* 28: 69–73, 2000.
19. **Schafers M, Sorkin LS, and Sommer C.** Intramuscular injection of tumor necrosis factor-alpha induces muscle hyperalgesia in rats. *Pain* 104: 579–588, 2003.
20. **Simons DG, Travell JG, and Simons PT.** *Travell and Simons' Myofascial Pain and Dysfunction: The Trigger Point Manual. Vol 1. Upper Half of Body* (2nd ed.). Baltimore, MD: Williams and Wilkins, 1999.
21. **Skyba DA, King EW, and Sluka KA.** Effects of NMDA and non-NMDA ionotropic glutamate receptor antagonists on the development and maintenance of hyperalgesia induced by repeated intramuscular injection of acidic saline. *Pain* 98: 69–78, 2002.
22. **Sluka KA, Kalra A, and Moore SA.** Unilteral intramuscular injections of acidic saline produce a bilateral, long-lasting hyperalgesia. *Muscle Nerve* 24: 37–46, 2001.
23. **Sluka KA, Rohlwing JJ, Bussey RA, Eikenberry SA, and Wilken JM.** Chronic muscle pain induced by repeated acid injection is reversed by spinally administered μ- and ∂-, but not κ-, opioid receptor agonists. *J Pharmacol Exp Ther* 302: 1146–1150, 2002.
24. **Yaksh TL.** Future advances in pain pharmacology: what does the present say about the future? *Proc West Pharmacol Soc* 45: 211–218, 2002.
25. **Yaksh TL, Yamamoto T, and Myers RR.** Pharmacology of nerve compression evoked hyperesthesia. In: *Hyperalgesia and Allodynia,* edited by Willis W. New York: Raven, 1995, p. 245–258.