VOCCs and TREK-1 ion channel expression in human tenocytes

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Submitted 6 February 2006; accepted in final form 27 September 2006

Magra M, Hughes S, El Haj AJ, Maffulli N. VOCCs and TREK-1 ion channel expression in human tenocytes. Am J Physiol Cell Physiol 292: C1053–C1060, 2007. First published October 11, 2006; doi:10.1152/ajpcell.00053.2006.—Mechanosensitive and voltage-gated ion channels are known to perform important roles in mechanotransduction in a number of connective tissues, including bone and muscle. It is hypothesized that voltage-gated and mechanosensitive ion channels also may play a key role in some or all initial responses of human tenocytes to mechanical stimulation. However, to date there has been no direct investigation of ion channel expression by human tenocytes. Human tenocytes were cultured from patellar tendon samples harvested from five patients undergoing routine total knee replacement surgery (mean age: 66 yr; range: 63–73 yr). RT-PCR, Western blotting, and whole cell electrophysiological studies were performed to investigate the expression of different classes of ion channels within tenocytes. Human tenocytes expressed mRNA and protein encoding voltage-operated calcium channel (VOCC) subunits (Ca α1A, Ca α1C, Ca α1D, Ca αβδ) and the mechanosensitive tandem pore domain potassium channel (2PK+) TREK-1. They exhibit whole cell currents consistent with the functional expression of these channels. In addition, other ionic currents were detected within tenocytes consistent with the expression of a diverse array of other ion channels. VOCCs and TREK channels have been implicated in mechanotransduction signaling pathways in numerous connective tissue cell types. These mechanisms may be present in human tenocytes. In addition, human tenocytes may express other channel currents. Ion channels may represent potential targets for the pharmacological management of chronic tendinopathies.

tendon; mechanosensitive; calcium; potassium; patch clamping; voltage-operated calcium channels

TENDONS ARE INTERPOSED between muscles and bones, transmit the forces produced by muscles to bones, and thus make joint motion possible. The anatomy of a tendon is critical for its physical properties (32, 35, 50). Tendons are rich in collagen and also contain glycosaminoglycans, noncollagenous proteins, cells, and water (44). Tenoblasts and tenocytes constitute about 90–95% of the cellular elements of tendons and align themselves in rows between collagen fiber bundles (31). Tenoblasts are highly metabolic immature spindle-shaped cells, with numerous cytoplasmic organelles that elongate and transform into mature tenocytes (31). Tenocytes are responsible for producing extracellular matrix proteins (5, 6, 33, 36).

The composition and microarchitecture of tendon tissue is continually adapted in response to changes in mechanical demands placed on the tissue (1). Overuse of tendons can lead to detrimental changes in tendon tissue structure and result in tendinopathic changes (61). Despite the clear clinical relevance of mechanotransduction signaling pathways in tenocytes, the mechanisms by which these cells perceive and respond to mechanical stimuli are poorly understood.

Calcium ions play an important role in mechanotransduction and act as one of the primary second messengers utilized by cells to convert mechanical signals to biochemical signals (8, 9). Preliminary data has shown upregulation of calcium signaling pathways in human tenocytes exposed to fluid flow-induced shear stress (19, 20, 23), and it has been proposed that mechanosensitive and voltage-gated ion channels may play a key role in the initial responses of human tenocytes to mechanical load (4, 19, 20, 23, 65). However, to date there has been no direct investigation of ion channel expression by human tenocytes.

Both mechanosensitive and voltage-gated ion channels have been allocated key roles in mechanotransduction signaling pathways in other connective tissues, including bone (17, 21, 41, 48, 60), smooth muscle (15, 29, 43), and heart cells (49). Voltage-operated calcium channels (VOCCs) permit the influx of extracellular calcium in response to change in membrane potential and form the basis of electrical signaling in excitable cells (10). VOCCs also are potentially mechanosensitive (43). Mechanosensitive members of the tandem pore domain potassium channel (2PK+) family (53, 54), including TREK-1 and TREK-2, may be involved in mechanotransduction signaling pathways in smooth muscle cells (37), heart cells (2, 24, 62), and bone cells (13, 30).

TREK-1 channels produce a spontaneously active background leak K+ conductance to hyperpolarize the cell membrane potential and regulate electrical excitability (28, 39, 53). TREK-1 channels are largely insensitive to traditional potassium channel blockers, including 4-aminopyridine (4-AP), tetraethylammonium ions (TEA), Cs+, and Ba2+ (25, 40, 54). TREK-1 is sensitive to membrane stretch (51), lysophosphatidyicholines (LPCs) (46, 54), lysophosphatidic acids (12), polyunsaturated fatty acids (51), intracellular pH (47), temperature (45), and a range of clinically relevant compounds including general and local anesthetics (26, 34, 52, 58).

In this study, we have used a combination of PCR, protein analysis, and whole cell electrophysiology to demonstrate the expression of VOCCs and TREK-1 ion channels in human tenocytes.

METHODS

Unless otherwise stated, all chemicals were obtained from Sigma (Gillingham, UK). The study was approved by the hospital’s Ethics Committee. Informed written consent was obtained from each patient.

Cell culture. Human tenocytes were grown from patellar tendon specimens harvested from five patients (mean age: 67 yr; range: 63–73 yr). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
63–73 yr) undergoing routine total knee replacement surgery at the University Hospital of North Staffordshire. Patellar tendon specimens (0.5 × 0.5 cm) were harvested from each patient during surgery and placed into a sterile container containing growth medium (DMEM, 10% vol/vol fetal calf serum, 1% vol/vol antibiotic and antifungal solution, and 2 mM L-glutamine). Tendon samples were then cultured in 25-cm² tissue culture flasks containing growth medium for 1 wk at 37°C with 5% CO₂. After this initial period, medium was replaced every 2–3 days and cells were passaged on reaching 95% confluence. All cells were used at passage 1.

Polymerase chain reaction. RT-PCR was used to investigate the expression of mRNA transcripts encoding the mechanosensitive 2P⁺ channels TREK-1, TREK-2, and TRAAK and also Ca₁α₁A, Ca₁α₁B, Ca₁α₁C, Ca₁α₁D, Ca₁β₁, VOCC subunits in human tenocytes from five patients. Total RNA was extracted from tenocytes using a modified guanidine thiocyanate method (55). RNA extracted from total human brain (BD Biosciences, Palo Alto, CA) was used as a positive control for the expression of TREK-1, TREK-2, TRAAK, Ca₁α₁A, Ca₁α₁B, Ca₁α₁C, Ca₁α₁D, and Ca₁β₁. All samples were treated with 1 μl of RNase-free DNase (Promega, Southampton, UK) to remove any traces of genomic DNA.

First-strand cDNA synthesis was performed using the SuperScript II kit (Invitrogen, Paisley, Scotland, UK) in accordance with the manufacturer’s guidelines. Oligonucleotide primers were designed to amplify human TREK-1, TREK-2, TRAAK, Ca₁α₁A, Ca₁α₁B, Ca₁α₁C, Ca₁α₁D, and Ca₁β₁ (primer sequences are shown in Table 1; cycling parameters are shown in Table 2). PCR products were resolved on 1% agarose gels containing 0.5 μg/ml ethidium bromide and viewed under ultraviolet light.

Western blotting. Whole cell lysates were prepared by resuspending human tenocytes in PBS-Trition (0.05%) at room temperature for 5 min, and total protein concentrations were determined using the Lowry colorimetric method (Sigma). Whole cell lysate (20 μg/lane) was mixed with an equal volume of loading buffer (0.2 mM Tris-HCl, pH 6.8, 3% wt/vol SDS, 30% vol/vol glycerol, 15% vol/vol acrylamide gels) as described previously (64). A standard protein ladder (Amersham Biosciences, Little Chalfont, UK) was used for determination of protein sizes. Immunostaining of nitrocellulose membranes was performed using standard immunochromatex-cence techniques as described previously (64). Membranes were incubated with polyclonal rabbit antibodies for 1 h (see Table 3) and then with a peroxidase-conjugated goat anti-rabbit IgG (1:20,000; Sigma) for 1 h. Detection of labeled proteins was performed using a chemiluminescence solution (Amersham Biosciences) and ECL-hyperfilm chemiluminescence detection paper (Amersham) and developed using standard photographic reagents (Ilford, London, UK).

Whole cell patch-clamp electrophysiology. Whole cell recordings were performed at room temperature (28–30°C) using a PC-501 patch-clamp amplifier (Warner, Hamden, CT) and WCP software (version 3.2; John Dempster, Strathclyde University, Glasgow, Scotland, UK). Glass microelectrodes were made from 1.5-mm-diameter filamented glass capillaries (Harvard Apparatus, Edenbridge, UK) and heat-polished to a resistance of 8–10 MΩ. Cells were seeded at low density and allowed to attach and spread for at least 4 h before recording. Only isolated cells without obvious cell-to-cell contacts were chosen for recordings.

To isolate Ca⁺⁺ currents, cells were bathed in BaCl₂ saline comprising 108 mM BaCl₂ and 10 mM HEPES (pH 7.6 with NaOH) (final sodium concentration: ~3 mM) (14), with internal pipette saline comprising 150 mM CsCl, 10 mM HEPES, 5 mM EGTA, and 10 mM D-glucose (pH 7.3 with CsOH) (3). For recordings of Ca⁺⁺ currents, a series of depolarizing steps (duration: 400 ms; increment: +20 mV) were applied from a holding potential of +60 mV, where depolarizing steps were interspersed with hyperpolarizing steps of one-quarter magnitude to enable P4 leak subtraction (7).

For isolation of TREK-1 currents, cells were bathed in either physiological saline comprising 140 mM NaCl, 3 mM KCl, 2.5 mM CaCl₂, and 10 mM HEPES (pH 7.6 with NaOH) or TEA-4 AP saline, where 23 mM of NaCl were replaced with 20 mM TEA and 3 mM 4-AP. The pipette saline comprised 140 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 11 mM EGTA, and 10 mM HEPES (pH 7.3 with KOH). Stimulus protocols included a voltage ramp from −100 to +100 mV (duration: 500 ms) and a series of depolarizing voltage steps (duration: 500 ms; increment: +10 mV) from −100 to +100 mV from a holding potential of 0 mV, chosen to reduce contamination by voltage-gated currents. In addition, leak subtraction was performed using the protocols described for the isolation of Ca⁺⁺ currents. Stocks of C:16 LPC (lyso-PAF) were dissolved in ethanol (5 mM) and diluted in physiological saline before being added directly to the external bath during whole cell recordings (final bath concentration: ~50 μM). All saline solutions were double filtered (0.2 μm) before use.

Table 1. Primer sequences

<table>
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<th>Oligonucleotide Primer</th>
<th>Primer Sequence 5’ to 3’</th>
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<tr>
<td>GAPDH forward</td>
<td>GATATTGAAGAATGGTTGTCAT</td>
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<tr>
<td>GAPDH reverse</td>
<td>TACACAGGACGCTTGCCCA</td>
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<tr>
<td>Ca₁α₁B reverse</td>
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<td>Ca₁α₁C reverse</td>
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Table 2. Cycling parameters for PCR

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<td>57°C</td>
<td>72°C</td>
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<tr>
<td>TREK-1</td>
<td>36</td>
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<td>Ca₁α₁D</td>
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<td>62°C</td>
<td>72°C</td>
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Table 3. Antibodies used for Western blotting

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<td>Rabbit polyclonal anti-GAPDH</td>
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<td>Abcam, Cambridge, UK</td>
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<tr>
<td>Rabbit polyclonal anti-TREK-1</td>
<td>1:800</td>
<td>Alomone Labs, Jerusalem, Israel</td>
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<tr>
<td>Rabbit polyclonal anti-Ca₁α₁A</td>
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</tr>
<tr>
<td>Rabbit polyclonal anti-Ca₁α₁D</td>
<td>1:200</td>
<td>Santa Cruz Biotechnology, Santa Cruz, CA</td>
</tr>
<tr>
<td>Rabbit polyclonal anti-Ca₁β₁</td>
<td>1:200</td>
<td>Alomone Labs, Jerusalem, Israel</td>
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</tbody>
</table>
Statistical analysis. All data are shown as means ± SE, and all statistical analysis was performed using Student’s t-test.

RESULTS

Polymerase chain reaction. Expression of the housekeeping gene GAPDH was used to validate reverse transcription and also to gauge equivalence of cDNA content between samples. A single PCR product of the expected size (611 bp) was detected in each case. All amplicon band intensities were similar, suggesting that cDNA content was comparable between specimens. No bands were observed in the RT-ve and PCR-blanks or in “no RT” controls performed on each sample, confirming that contaminating genomic DNA was not present. As expected, expression of TREK-1, TREK-2, TRAAK, Ca\(\alpha_{1A}\), Ca\(\alpha_{1B}\), Ca\(\alpha_{1C}\), Ca\(\alpha_{1D}\), and Ca\(\alpha_{2}\delta_{1}\) was detected in total human brain cDNA. In each case, the amplicon size was as expected from the published coding mRNA sequence (340, 240, 182, 230, 206, 502, 289, and 448 bp, respectively; NCBI Database, http://www.ncbi.nlm.nih.gov/).

Expression of TREK-1, Ca\(\alpha_{1A}\), Ca\(\alpha_{1C}\), Ca\(\alpha_{1D}\), and Ca\(\alpha_{2}\delta_{1}\) mRNA was detected in human-derived tenocytes (n = 5; Fig. 1). TREK-1 and Ca\(\alpha_{2}\delta_{1}\) typically showed strong expression, whereas levels of Ca\(\alpha_{1A}\) and Ca\(\alpha_{1C}\) expression were more variable. For all five patients, expression of Ca\(\alpha_{1D}\) mRNA was relatively low compared with whole human brain controls. mRNA transcripts encoding TREK-2, TRAAK, and Ca\(\alpha_{1B}\) were not detected in human tenocyte samples (data not shown).

Western blotting. Western blotting of whole cell protein extracts was performed to investigate the expression of TREK-1, Ca\(\alpha_{1A}\), Ca\(\alpha_{1C}\), Ca\(\alpha_{1D}\), and Ca\(\alpha_{2}\delta_{1}\) protein subunits in human tenocytes via Western blotting. Whole cell protein (20 μg) was loaded for each sample. MWM, molecular mass marker; lanes 1–5, protein from tendon samples from individual patients.

Western blotting demonstrated the expression of TREK-1, Ca\(\alpha_{1A}\), Ca\(\alpha_{1D}\), and Ca\(\alpha_{2}\delta_{1}\) protein in human tenocytes. Western blotting of GAPDH protein was performed to validate equality of protein loading for each sample.

Western blotting of Ca\(\alpha_{1A}\) and Ca\(\alpha_{1B}\) was not detected in human tenocyte samples (data not shown).

Whole cell electrophysiology. Whole cell recordings were performed on 105 cells derived from 3 different patients. The number of cells patched under each set of saline conditions is shown in Table 4.

L-type VOCCs. Ca\(^{2+}\) currents were isolated using barium as the charge carrier. In the absence of BAY K, no inward
currents were detected in human tenocytes patched in BaCl₂ salines (10 or 108 mM BaCl₂ saline; n = 19 cells). After the addition of the L-type VOCC agonist BAY K (500 nm–1 μM) to the external saline solution, inward currents were observed in a small number of cells (3 of 47 cells). These inward currents were typically small in magnitude but did demonstrate clear voltage-dependent activity consistent with the expression of L-type VOCCs (Fig. 3, A and B). The peak inward currents recorded from cells bathed in 10 and 108 mM BaCl₂ (with 500 nM and 1 μM BAY K) were observed at around +10 and +20 mV respectively, consistent with the well-documented effects of barium ions and BAY K on membrane depolarization and L-type VOCC activation kinetics (27, 64).

TREK-type currents. In addition to inward currents, a number of cells bathed in BaCl₂ saline exhibited a spontaneously active “leak-type” K⁺ current with similarities to currents elicited by the 2PK⁺ channel family, particularly TREK-1 type currents (Fig. 4). These currents were spontaneously active at all membrane potentials tested (−100 to +100 mV), showed strong outward rectification, and showed no signs of classic time-dependent or voltage-dependent inactivation (Fig. 4, A and B). The reversal potential of these currents was typically in the region of −30 to −90 mV (Fig. 4A) and is therefore consistent with significant conduction of potassium ions (presumably remaining within the cell following attainment of the whole cell configuration). Isolation of the rectification-dependent component of the outward currents (leak subtracted) again revealed currents with similar characteristics (Fig. 4C).

In 108 mM BaCl₂ saline, this current was detected in 33 of 56 cells. The mean leak-subtracted outward currents recorded at +80 mV were 38.7 ± 5.58 pA. This outward current was also detected in 5 of 10 cells patched in 10 mM BaCl₂ saline, where the mean leak-subtracted current observed at +80 mV was 27.54 ± 7.53 pA (Fig. 4D).

Table 4. Whole cell patching under different saline conditions

<table>
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<tr>
<th>Saline Condition</th>
<th>n</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>108 mM BaCl₂</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>108 mM BaCl₂, 1 μM BAY K</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>10 mM BaCl₂, 500 nM BAY K</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>108 mM BaCl₂, 500 nM BAY K, no FCS</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>NaCl</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>NaCl-TEA</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>NaCl-TEA-4-AP</td>
<td>23</td>
<td>2</td>
</tr>
</tbody>
</table>

The combined presence of both TEA and 4-AP previously has been used to isolate TREK-1 currents from other native K⁺ currents in mouse striatal neurones (28), and thus this approach was used to confirm the presence of TREK-type currents in human tenocytes. Whole cell recordings from human tenocytes patched in NaCl-based salines containing 20 mM TEA and 3 mM 4-AP again revealed the presence of a spontaneously active, outwardly rectifying noninactivating K⁺ current with similarities to TREK-type currents. This current was the dominant current in 9 of 23 cells. The mean outward current observed at +80 mV was 37.98 ± 6.19 pA (leak subtracted). The removal of 4-AP, or both TEA and 4-AP, from the saline solution had little effect on the magnitude of these TREK-like currents, with mean currents observed at +80 mV of 42.23 ± 9.18 pA (3 of 8 cells) and 32.75 (2 of 8 cells), respectively (leak subtracted) (Fig. 4D).

The TREK subfamily of the 2PK⁺ family are the only mammalian potassium channels to show sensitivity to the external application of anionic LPCs (46, 54). The addition of LPC (50 μM C:16 lyso-PAF) to the external saline solution (NaCl saline containing 20 mM TEA and 3 mM 4-AP) produced a clear, reversible increase in the TREK-type currents recorded from human tenocytes (n = 6). The mean increase in current was 220%, from 58.6 ± 16.6 to 146.01 ± 30.64 pA (before leak subtraction), and 167.8%, from 39.7 ± 13.15 to 83.97 ± 25.81 pA (using leak subtraction). The LPC-induced current shared characteristics and biophysical properties with TREK-type currents recorded before the addition of LPC. LPC-induced currents showed clear outward rectification, showed no signs of time-dependent inactivation, and had a reversal potential similar to that before the addition of LPC (typically −65 to −90 mV) (Fig. 5).

**DISCUSSION**

This study demonstrates that human tenocytes express mRNA and protein for a number of different VOCC channel subunits. Human tenocytes express mRNA transcripts for Caα1A, Caα1C, and Caα1D VOCC pore-forming units and also the Caα2δ1 auxiliary subunit. Caα1C and Caα1D subunits encode VOCCs with high voltage activation thresholds, termed L-type VOCCs, whereas Caα1A units produce P/Q-type channels (10, 18). In addition to VOCCs, human tenocytes also express mRNA and protein encoding the mechanosensitive 2PK⁺ channel TREK-1 but not TREK-2 or TRAAK.

This study is the first to use whole cell electrophysiology to directly investigate the expression of ion channels within...
human tenocytes. Whole cell patch-clamp recordings demonstrate that these cells express ion channel currents with similarities to both L-type VOCCs and TREK-1.

BaCl₂-based salines were used to isolate Ca²⁺ currents (14), and whole cell recordings performed under these conditions revealed the presence of voltage-activated inward currents in a small number of cells. These currents showed activation kinetics consistent with the expression of L-type VOCCs, and the fact that these currents were only observed in the presence of the L-type agonist BAY K further suggests that they originate from the expression of L-type VOCCs. The finding that human tenocytes express L-type VOCCs confirms previous reports that increased levels of intracellular calcium observed in tenocytes following the application of mechanical strain could

Fig. 4. Whole cell patch-clamp recordings from a human tenocyte in 108 mM BaCl₂ saline. A: whole cell "ramp" recording. Top trace shows the whole cell current recorded in response to a 500-ms voltage ramp from −100 to +100 mV from a holding potential of 0 mV (shown by bottom trace). Top trace shows outwardly rectifying current-voltage (I-V) relationship and reversal potential around −50 mV. B: non-inactivating nature of whole cell current recorded from the same cell in response to a series of 500-ms +10-mV voltage steps from −100 to +100 mV from a holding potential of 0 mV. C: whole cell current that remains after the use of a standard leak subtraction protocol, following a series of depolarizing +20-mV steps from −60 to +100 mV from a holding potential of −60 mV. D: summary of the mean "TREK-like" current recorded from tenocytes under varying saline conditions in the presence of the traditional potassium channel blockers TEA, 4-aminopyridine (4-AP), Ba²⁺, and Cs⁺. Ch.0 represents recorded current, Ch.1 represents applied voltage.

Fig. 5. A: a series of consecutive ramp recordings from an individual tenocyte before and after the addition of 50 μM lysophosphatidylcholine (LPC, C16: lyso-PAF) to the external saline. B: summary of effect of LPC on TREK-type currents (n = 6, data shown are non-leak subtracted). *P < 0.01. C and D: leak-subtracted recordings before (C) and after (D) the application of LPC to the bathing saline (recordings from a different cell from that shown in A). Ch.0 represents recorded current, Ch.1 represents applied voltage.
partly be due to the entry of extracellular calcium via VOCCs (19, 20, 65).

Despite the relatively strong expression of L-type VOCCs channels indicated by mRNA and protein analysis, the VOCC currents recorded from tenocytes were small in magnitude and were detected in only a small percentage of cells. The low levels of VOCC currents recorded from tenocytes are, however, comparable with levels observed in other connective tissues, such as bone cells (14, 27). It is likely that levels of VOCC expression in tenocytes may be influenced by a number of factors, including time in culture (14, 56), serum concentrations and state of cell cycle (42), and exposure to hormones (57, 27). It also is possible that the activity of L-type VOCCs is downregulated or controlled by some form of inhibitory cell signaling, although further investigation is needed to clarify this point.

Whole cell recordings from human tenocytes also revealed the presence of spontaneously active, noninactivating outwardly rectifying K+ currents with similarities to TREK-1 currents. The TREK-type channels recorded from human tenocytes were relatively insensitive to combinations of external TEA (20 mM) and 4-AP (3 mM), external Ba2+ (10 and 108 mM), and internal Cs+ (150 mM) and was stimulated by the external application of LPC (50 μM C16 lyso-PAF), a feature unique to the TREK-1, TREK-2, and TRAAK subfamily of mammalian potassium ion channels (53, 54). These currents were observed from a holding potential of 0 mV, in the absence of ATP or cGMP in the pipette saline, and with intracellular calcium buffered to minimize any contamination of records by calcium-activated K+ currents.

The biophysical properties of the TREK-type currents recorded from human tenocytes and their responses to LPC are consistent with previous published reports of cloned and native TREK-1 channels (46, 54) and are indistinguishable from whole cell currents recorded from COS-7 cells transiently transfected with murine TREK-1 channels under similar conditions in our laboratory (data not shown) (30). We conclude that the whole cell currents observed from human tenocytes originate, at least in part, from the expression of TREK-1 channels.

TREK-1 is a spontaneously active background leak channel, and thus the activity of these channels is likely to contribute to the resting membrane potentials of tenocytes, potentially influencing levels of cell proliferation (30). TREK-1 is sensitive to both membrane stretch and fluid flow-induced shear stress (37, 47, 51) and has been implicated in mechanotransduction signaling pathways in smooth muscle cells (37), heart cells (2, 24, 62), and bone cells (30). It is therefore possible that TREK-1 may perform a direct role in mechanosensing by tenocytes. TREK-type channels (TREK-2) were recently associated with calcium-independent mechanical load responses in osteoblasts (13). It thus may be possible that TREK channels may mediate calcium-independent responses within tenocytes.

Furthermore, TREK-1 channels are modulated by a diverse array of signaling molecules involved in mechanotransduction signaling pathways within other connective tissues, including cAMP (22, 45), nitric oxide (37), glutamate (11, 38), MAP kinases (2), prostaglandins (45), and diacylglycerol (11). Interaction of these signaling molecules with TREK-1 presents a series of potential pathways by which mechanical stimulation of tenocytes may lead to secondary changes in membrane potential and regulation of downstream signaling events.

In addition to L-type VOCCs and TREK-type currents, a number of other whole cell currents were recorded from human tenocytes, consistent with the expression of a diverse array of other ion channels, including voltage-gated K+ currents, inward Na+ currents, and a number of other leak-type currents. However, although the precise identity of the channels underlying these currents remains unclear at present, it is clear that these different classes of ion channels may act in combination to regulate many aspects of tenocyte function, including responses to physical exercise.

**Clinical significance.** Our results from RT-PCR, Western blotting, and whole cell electrophysiological studies demonstrate that human tenocytes express VOCCs and TREK-1 channels. This is the first study to demonstrate the presence of functional ion channels on human tenocytes. Malfunctioning ion channels may lead to tendinopathic changes. In osteoblasts, desensitization occurs during prolonged periods of continuous mechanical loading (16, 59), and this phenomenon was recently highlighted as a potential target for clinical therapies (63). A similar approach could be applied to tenocytes, and TREK-1 and VOCC channels could be potential targets for pharmacological management of tendinopathies.

In conclusion, human tenocytes express a diverse array of ion channels, including L-type VOCCs and TREK-1. VOCCs are likely to be a key mediator of calcium signaling events in human tenocytes, whereas TREK-1 could potentially perform a number of roles in tenocytes, ranging from osmoregulation and cell volume control, control of resting membrane potentials of electrical excitability, and the direct detection of mechanical stimuli. TREK-1 and VOCC channels could be potential targets for pharmacological management of chronic tendinopathies.

**ACKNOWLEDGMENTS**

We thank Leanne Cioni, Julia Magnay, and Karen Hampson for all help in this project.

**GRANTS**

This work was supported by The North Staffordshire Nuffield Hospital Charity.

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