Stretch-induced activation of \( \text{Ca}^{2+} \)-activated \( \text{K}^+ \) channels in mouse skeletal muscle fibers

NORA MALLOUK AND BRUNO ALLARD

Laboratoire de Physiologie des Eléments Excitables, Unité Mixte de Recherche, Centre National de la Recherche Scientifique 5578, Université C. Bernard Lyon I, 69622 Villeurbanne Cedex, France

Mallouk, Nora, and Bruno Allard. Stretch-induced activation of \( \text{Ca}^{2+} \)-activated \( \text{K}^+ \) (\( \text{K}_{\text{Ca}} \)) channels in mouse skeletal muscle fibers. Am. J. Physiol. Cell Physiol. 278: C473–C479, 2000.—High-conductance \( \text{Ca}^{2+} \)-activated \( \text{K}^+ \) (\( \text{K}_{\text{Ca}} \)) channels were studied in mouse skeletal muscle fibers using the patch-clamp technique. In inside-out patches, application of negative pressure to the patch induced a dose-dependent and reversible activation of \( \text{K}_{\text{Ca}} \) channels. Stretch-induced increase in channel activity was found to be of the same magnitude in the presence and in the absence of \( \text{Ca}^{2+} \) in the pipette. The dose-response relationships between \( \text{K}_{\text{Ca}} \) channel activity and intracellular \( \text{Ca}^{2+} \) and between \( \text{K}_{\text{Ca}} \) channel activity and membrane potential revealed that voltage and \( \text{Ca}^{2+} \) sensitivity were not altered by membrane stretch. In cell-attached patches, in the presence of high external \( \text{Ca}^{2+} \) concentration, stretch-induced activation was also observed. We conclude that membrane stretch is a potential mode of regulation of skeletal muscle \( \text{K}_{\text{Ca}} \) channel activity and could be involved in the regulation of muscle excitation during contraction-relaxation cycles.

mechanosensitivity; patch clamp; contraction

HIGH-CONDUCTANCE \( \text{Ca}^{2+} \)-activated \( \text{K}^+ \) (\( \text{K}_{\text{Ca}} \)) channels are found in the plasma membranes of different cell types and are implicated in the regulation of a variety of functions, such as cell firing in neurons, secretion in endocrine and exocrine cells, and myogenic tone in arterial smooth muscle (for review, see Refs. 4, 12, 14, 16, 27). The basic characteristic of these channels is that their opening is induced by an increase in intracellular \( \text{Ca}^{2+} \) concentration ([\( \text{Ca}^{2+} \)]) as well as membrane depolarization. In mammalian skeletal muscle, \( \text{K}_{\text{Ca}} \) channels have been extensively studied in excised patches from cultured and adult muscles and in tubular vesicles incorporated into planar bilayers (e.g., Refs. 3, 15, 28), but their role has not been yet clearly defined. Nevertheless, conditions that may lead to the opening of \( \text{K}_{\text{Ca}} \) channels in their cellular environment were recently revealed. Allard et al. (1) showed that, in depolarized skeletal muscle fibers, the increase in intracellular [\( \text{Ca}^{2+} \)] resulting from \( \text{Ca}^{2+} \) entry through end plate \( \text{ACh} \) receptors could induce \( \text{K}_{\text{Ca}} \) channel opening. Additionally, after recording single-channel activity from a voltage-clamped portion of muscle fiber, \( \text{K}_{\text{Ca}} \) channels were activated by a rise in intracellular [\( \text{Ca}^{2+} \)] following long-lasting voltage-activated sarcoplasmic reticulum \( \text{Ca}^{2+} \) release. In both studies, intracellular \( \text{Ca}^{2+} \) measurements revealed a dissociation between the presumed submembranous [\( \text{Ca}^{2+} \)] estimated from \( \text{K}_{\text{Ca}} \) channel activity and the average cytoplasmic [\( \text{Ca}^{2+} \)]. Such a discrepancy suggested that, in situ, factors associated with the intracellular environment or changes in the properties of the channel protein itself may increase the \( \text{K}_{\text{Ca}} \) channel open probability (\( \text{P}_o \)). One candidate is the membrane deformation induced by the small contractile response associated with the increase in intracellular [\( \text{Ca}^{2+} \)], which may alter \( \text{K}_{\text{Ca}} \) channel activity; activation of \( \text{K}_{\text{Ca}} \) channels by membrane stretch has indeed been demonstrated in another type of contractile cell, i.e., in vascular smooth muscle cells (13). However, in skeletal muscle, Guharay and Sachs (8), who were the first to report the existence of stretch-activated ion channels, indicated that \( \text{K}_{\text{Ca}} \) channels were not sensitive to membrane stretch in cultured embryonic chick skeletal muscle. In the present study, we reinvestigate the mechanosensitivity of \( \text{K}_{\text{Ca}} \) channels in adult mammalian skeletal muscle. We demonstrate that skeletal muscle \( \text{K}_{\text{Ca}} \) channel activity is enhanced by membrane stretch. We also examine the effects of membrane stretch on the \( \text{Ca}^{2+} \) and voltage dependence of \( \text{K}_{\text{Ca}} \) channels. Our findings suggest that, during muscle activation, the membrane deformation associated with the increase in intracellular [\( \text{Ca}^{2+} \)] and membrane depolarization constitutes a potential mode of regulation of \( \text{K}_{\text{Ca}} \) channel opening.

METHODS

Isolation of skeletal muscle fibers. Mice (C57BL/10ScSn) were killed by cervical dislocation following isoflurane anesthesia. Isolated skeletal muscle cells were obtained from the flexor digitorum brevis and intersseal muscles by a classic enzymatic dissociation process; muscles were incubated for 1 h at 37°C in a Tyrode solution containing collagenase (2 mg/ml; Sigma type I). After enzyme treatment, muscles were rinsed with Tyrode solution and stored in Tyrode solution at 4°C until use. Intact skeletal muscle fibers were separated from the muscle mass by gently triturating the muscle with a plastic Pasteur pipette. All experiments were carried out at room temperature (20–23°C).

Electrophysiology. Single-channel currents were recorded from cell-attached or inside-out membrane patches using a
patch-clamp amplifier (model RK 400, Bio-Logic, Claix, France). Currents flowing into the pipette were considered to be positive. Currents were digitized and stored on disk using an analog-to-digital converter (Lab Master DMA board, Scientific Solutions, Salom, OH) controlled by the Biopatch-Acquire software (Bio-Logic). Channel activity was determined from the average current (\(I_o\)) as \(NP_o = \frac{I_o}{t}\) in each patch, where \(N\) is the number of channels in the patch, and \(P_o\) is the open state probability. Pipettes were pulled from borosilicate glass capillaries and had resistances of 3–4 M\(\Omega\) when filled with Tyrode and immersed in the K\(^{+}\)-rich bathing solution. This corresponds to a tip diameter of \(\sim 0.7–1.1\ \mu m\) (24). Care was taken to use gentle patches; positive pressure was applied to the pipette before the pipette had entered the bath and until the pipette was in contact with the cell. Usually, the release of positive pressure from the pipette was sufficient to form a gigaseal. If not, a slight depression of the pipette, lasting <1–2 s and of far less amplitude than the one applied to induce a detectable stretch activation of K\(_{Ca}\) channels, was enough to form the gigaseal.

Results are given as means \(\pm SE\). Channel activities were statistically analyzed using Student’s unpaired t-test. Values were considered significant when \(P < 0.05\).

Solutions and chemicals. External Tyrode solution contained (in mM) 140 NaCl, 5 KCl, 1 MgCl\(_2\), 2.5 CaCl\(_2\), or 0 Ca\(^{2+}\) and 1 EGTA when mentioned), and 10 HEPES, adjusted to pH 7.4 with NaOH. For inside-out experiments, pipettes were filled with Tyrode solution, and the cytoplasmic face of membrane patches was exposed to an internal K\(^{+}\)-rich solution containing (in mM) 140 KCl, 0.01 CaCl\(_2\), 1 MgCl\(_2\), 10 HEPES, and 1 mM ATP, adjusted to pH 7.2 with KOH. In some inside-out experiments (see Figs. 3 and 4), calibration solutions were used to control the free [Ca\(^{2+}\)] at the cytoplasmic face of membrane patches. Calibration solutions were prepared from two stock solutions containing (in mM) 100 KCl, 10 EGTA, and 10 PIPES at pH 7.00 with and without 10 CaCl\(_2\), respectively. Various concentrations of free Ca\(^{2+}\) were achieved by mixing these two stock solutions in different ratios. In cell-attached experiments, pipettes were filled with a Ca\(^{2+}\)-rich Tyrode solution containing (in mM) 140 NaCl, 5 KCl, 50 CaCl\(_2\), 1 MgCl\(_2\), and 10 HEPES, adjusted to pH 7.4 with NaOH; fibers were bathed in an external K\(^{+}\)-rich solution containing (in mM) 140 KCl, 50 CaCl\(_2\), 1 MgCl\(_2\), and 10 HEPES, adjusted to pH 7.4 with KOH. This bathing solution produced neither contracture nor detectable signs of cell deterioration.

ATP (potassium salt; Sigma) was buffered at pH 7 (adjusted with KOH), stored in aliquots at \(-20^\circ C\), and diluted to the required concentrations in the perfused solutions.

Membrane patches were exposed to different solutions by placing them in the mouth of a perfusion tube from which flowed the rapidly exchanged solutions. Flow of the solutions did not affect channel activity.

Membrane stretch was elicited by applying negative pressure to the back end of the patch pipette through the suction port of the pipette holder. The pressure level was established by monitoring the height of water in a U-shaped tube, one end of which was at room air pressure and the other end of which was at the desired pressure, switchable to the pipette. Step changes in pressure were produced with a manually operated valve system that switched the pipette holder between the U tube and room air.

RESULTS

Upon patch excision from skeletal muscle fibers (Tyrode solution in the pipette; 1 mM ATP and 10 µM Ca\(^{2+}\) in the bath), one type of channel was spontaneously active at 0 mV. These channels were identified as high-conductance K\(_{Ca}\) channels, as described in previous studies under similar experimental conditions (1, 10), on the basis of their conductance (\(-60\) pS at 0 mV) and the dependence of their activity on intracellular Ca\(^{2+}\). Figure 1 shows the effects of membrane stretch on K\(_{Ca}\) channel activity in inside-out patches. In Fig. 1A, one K\(_{Ca}\) channel was present in the patch, as revealed by increasing the cytoplasmic face [Ca\(^{2+}\)] to 2.5 mM (not shown). Application of negative pressure of \(-4\) kPa reversibly activated the channel. In this patch, NP\(_o\) was changed from 0.06 under control conditions to 0.15 during pressure application. In the patch illustrated in Fig. 1B, three or, very rarely, four steps of channel activity were detected under control conditions. Suction induced a sustained activation of the channels, and four channels were frequently active together; NP\(_o\) increased from 1.45 to 2.5. While nega-

---

**Fig. 1.** Stretch activation of Ca\(^{2+}\)-activated K\(^{+}\) (K\(_{Ca}\)) channels in inside-out patches from skeletal muscle fibers. In 3 patches illustrated, cytoplasmic face was exposed to an intracellular solution containing 1 mM ATP and 10 µM Ca\(^{2+}\), and patch was held at 0 mV. In A and B, pipettes were filled with Tyrode. In A, horizontal bar indicates period of pressure application. In B, upper horizontal bar indicates period of pressure application, and lower bar indicates period during which an intracellular solution containing 1 mM EGTA and no added Ca\(^{2+}\) was applied. In C, pipette was filled with a Tyrode solution containing 1 mM EGTA and no added Ca\(^{2+}\), and bar indicates period of pressure application. The product of number of channels in patch (N) and open state probability (P\(_o\)) increased from 1.3 to 2.
tive pressure was constantly applied, removal of Ca\textsuperscript{2+} from the cytoplasmic face totally suppressed channel opening. This indicates that channels activated by negative pressure correspond to K\textsubscript{Ca} channels and that suction alone is insufficient to activate the channels. Additionally, single-channel current measurements indicated that the unitary conductance of the channel was not changed by membrane stretch.

A set of experiments was then performed in the absence of Ca\textsuperscript{2+} at the external face of the membrane to test the possibility that the increase in channel activity observed in response to membrane stretch could be the consequence of an influx of Ca\textsuperscript{2+} via stretch-activated channels or stretch-induced Ca\textsuperscript{2+} leak. Figure 1C shows that, in the absence of Ca\textsuperscript{2+} in the pipette, suction reversibly increased K\textsubscript{Ca} channel activity. On average, −4 kPa suction produced a 2.9 ± 0.75-fold increase in activity (n = 12) compared with 2.7 ± 0.5-fold (n = 14) with normal [Ca\textsuperscript{2+}] in the pipette. These values were not significantly different (P = 0.82). It is thus unlikely that the increase in channel activity could be secondary to an influx of Ca\textsuperscript{2+}.

At a given membrane potential and intracellular [Ca\textsuperscript{2+}], K\textsubscript{Ca} channels were found to be activated in a pressure-dependent manner. Figure 2 illustrates K\textsubscript{Ca} channel activity at different negative pressures in a patch held at 0 mV (Tyrode in the pipette and bath solution as above). In this patch, N\textsubscript{P0} was 0.07 in control and increased to 0.25, 0.4, and 1.1 in the presence of −2, −4, and −6 kPa negative pressure, respectively. The relationship between K\textsubscript{Ca} channel activity and negative pressure level is illustrated in Fig. 2B. The relationship was fitted with a Boltzmann equation: factor of increase of N\textsubscript{P0} = 1/[1 + e\textsuperscript{(P\textsubscript{o} − P)/k}], where P is the pressure applied to the pipette, P\textsubscript{o} is the amount of pressure required to induce half-maximal potentiation, and k is the steepness of the relation. The best fit to the mean data was obtained with values of 3.6 kPa for P\textsubscript{o} and 1.6 kPa for k. Maximum activation occurred at about −6 kPa.

To evaluate the relationships between membrane stretch and the different modes of K\textsubscript{Ca} channel activation, we examined the influence of membrane stretch on Ca\textsuperscript{2+} and voltage dependence of K\textsubscript{Ca} channels. In the first set of experiments (Fig. 3), membrane currents were recorded at 0 mV, and membrane patches were exposed in a cumulative manner to internal solutions devoid of ATP and containing increasing concentrations of free Ca\textsuperscript{2+}. Figure 3A shows segments of current recordings from a patch exposed to internal solutions containing 1.7, 3, and 4.4 µM Ca\textsuperscript{2+}, respectively. In the presence of 1.7 µM Ca\textsuperscript{2+}, a low activity of channels that, on the basis of their conductance (~20 pS under these ionic conditions), likely correspond to ATP-dependent K\textsuperscript{+} channels (K\textsubscript{ATP}; see Ref. 2) was detected, whereas no K\textsubscript{Ca} channel opening was observed. With increase of free [Ca\textsuperscript{2+}], K\textsubscript{Ca} channels gradually activated, and N\textsubscript{P0} was 0.55 and 1.5 in the presence of 3 and 4.4 µM Ca\textsuperscript{2+}, respectively. At each [Ca\textsuperscript{2+}] tested, application of negative pressure pulses of an amplitude of −4 kPa induced a reversible increase in channel opening. In response to membrane stretch, K\textsubscript{Ca} channel activity became just detectable in the presence of 1.7 µM Ca\textsuperscript{2+} and was augmented by factors of 1.9 and 1.8 in the presence of 3 and 4.4 µM Ca\textsuperscript{2+}, respectively. Additionally, the current trace obtained in the presence of 1.7 µM Ca\textsuperscript{2+} clearly shows that K\textsubscript{ATP} channel activity was not altered by membrane stretch. It should be noted that, in all of the patches tested displaying K\textsubscript{ATP} channel opening, we never saw any apparent modification in K\textsubscript{ATP} channel activity in response to membrane stretch. Figure 3B shows the relationship between K\textsubscript{Ca} channel activity and internal [Ca\textsuperscript{2+}] in the absence and in the presence of negative pressure of −4 kPa in the pipette, respectively. The increase in channel activity with intracellular [Ca\textsuperscript{2+}] in both conditions was fitted by a Hill
equation: \( NP_o = \frac{[\text{Ca}^{2+}]^N}{K^N + [\text{Ca}^{2+}]^N} \), where \( K \) is the [Ca\(^{2+}\)] producing half-maximal activity and \( N \) is the Hill coefficient. The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

Activation of \( K_{Ca} \) channel by membrane stretch could also be observed under certain conditions in cell-attached membrane patches. As previously reported (1), \( K_{Ca} \) channel activity was rarely detected in cell-attached patches established on resting cells bathed in

equation: \( NP_o = \frac{[\text{Ca}^{2+}]^N}{K^N + [\text{Ca}^{2+}]^N} \), where \( K \) is the [Ca\(^{2+}\)] producing half-maximal activity and \( N \) is the Hill coefficient. The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

Figure 4A shows current traces from an excised patch (4.4 \( \mu \)M free Ca\(^{2+}\) at the cytoplasmic face) at different membrane potentials. A low activity of \( K_{Ca} \) channels was observed at \(-20 \) mV, and channel opening was progressively increased with depolarization of the membrane patch. \( NP_o \) was 0.2, 1.5, and 2.3 at \(-20 \), 0, and \(+20 \) mV, respectively. At each membrane poten-

equation: \( NP_o = \frac{[\text{Ca}^{2+}]^N}{K^N + [\text{Ca}^{2+}]^N} \), where \( K \) is the [Ca\(^{2+}\)] producing half-maximal activity and \( N \) is the Hill coefficient. The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

Figure 4A shows current traces from an excised patch (4.4 \( \mu \)M free Ca\(^{2+}\) at the cytoplasmic face) at different membrane potentials. A low activity of \( K_{Ca} \) channels was observed at \(-20 \) mV, and channel opening was progressively increased with depolarization of the membrane patch. \( NP_o \) was 0.2, 1.5, and 2.3 at \(-20 \), 0, and \(+20 \) mV, respectively. At each membrane poten-

equation: \( NP_o = \frac{[\text{Ca}^{2+}]^N}{K^N + [\text{Ca}^{2+}]^N} \), where \( K \) is the [Ca\(^{2+}\)] producing half-maximal activity and \( N \) is the Hill coefficient. The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

Figure 4A shows current traces from an excised patch (4.4 \( \mu \)M free Ca\(^{2+}\) at the cytoplasmic face) at different membrane potentials. A low activity of \( K_{Ca} \) channels was observed at \(-20 \) mV, and channel opening was progressively increased with depolarization of the membrane patch. \( NP_o \) was 0.2, 1.5, and 2.3 at \(-20 \), 0, and \(+20 \) mV, respectively. At each membrane poten-

equation: \( NP_o = \frac{[\text{Ca}^{2+}]^N}{K^N + [\text{Ca}^{2+}]^N} \), where \( K \) is the [Ca\(^{2+}\)] producing half-maximal activity and \( N \) is the Hill coefficient. The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

The best fit to the mean data was obtained with values of 7 \( \mu \)M for \( K \) and 2.5 for \( N \) in the absence of pressure and with 6.6 \( \mu \)M for \( K \) and of 2.2 for \( N \) in the presence of negative pressure of \(-4 \) kPa.

Figure 4A shows current traces from an excised patch (4.4 \( \mu \)M free Ca\(^{2+}\) at the cytoplasmic face) at different membrane potentials. A low activity of \( K_{Ca} \) channels was observed at \(-20 \) mV, and channel opening was progressively increased with depolarization of the membrane patch. \( NP_o \) was 0.2, 1.5, and 2.3 at \(-20 \), 0, and \(+20 \) mV, respectively. At each membrane poten-
a K\(^{+}\)-rich solution containing 2.5 mM Ca\(^{2+}\), even at highly depolarized membrane potentials, presumably because of the too low concentration of free Ca\(^{2+}\) inside the cells under these experimental conditions. In these conditions, application of negative pressure of increasing amplitude to the point of rupture of the patch membrane never induced K\(_{\text{Ca}}\) channel opening. In mouse skeletal muscle fibers, elevated external [Ca\(^{2+}\)] (36 mM) increases intracellular Ca\(^{2+}\) levels near the sarcolemma (26). We thus used a medium containing 50 mM Ca\(^{2+}\) for the bath and intrapipette solutions; under these conditions, we expected the subsarcolemmal Ca\(^{2+}\) to reach a concentration high enough to induce detectable K\(_{\text{Ca}}\) channel opening. Additionally, the patch potential was depolarized to favor K\(_{\text{Ca}}\) channel opening. Figure 5 shows the effect of membrane stretch on channel activity in a cell-attached patch at a pipette potential of \(-40\) mV in the presence of an external K\(^{+}\)-rich solution in the bath and a Tyrode solution in the pipette, both containing 50 mM Ca\(^{2+}\). Suction increased the activity of channels that could be identified as K\(_{\text{Ca}}\) channels on the basis of their conductance (90 pS at +40 mV; see Fig. 5, inset); \(-4\) kPa reversibly increased NP\(_{\infty}\) from 0.04 to 0.34. A second application of pressure again potentiated channel activity. Similar results were obtained in two additional patches.

**DISCUSSION**

In this paper, we show that, in addition to the regulation by voltage and Ca\(^{2+}\), skeletal muscle K\(_{\text{Ca}}\) channels are also subject to regulation by membrane stretch. We demonstrate that suction of the membrane through the patch pipette gave rise in excised membrane patches to a reversible increase in channel activity in a dose-dependent manner. There is strong evidence that membrane stretch directly affects the skeletal muscle K\(_{\text{Ca}}\) channel protein itself or a membrane component closely related to the channel, since 1) stretch activation could be revealed in the absence of Ca\(^{2+}\) at the external face of the membrane and 2) little enzymatic machinery is thought to be present in excised membrane patches, ruling out a possible transduction via intracellular second messenger systems or cytosolic factors. Additionally, the fact that stretch activation was observed in excised patches makes it unlikely that membrane stretch recruits new channels; rather, it likely increases channel P\(_{\infty}\). This conclusion is strengthened by the fact that stretch activation occurred in patches containing one channel (see Fig. 1A).

In a previous study, Guharay and Sachs (8) reported that K\(_{\text{Ca}}\) channels were not affected by membrane stretch in skeletal muscle. However, these authors worked on tissue-cultured avian skeletal muscle, and it can be postulated that the difference between their results and ours may be due to the different types and/or to the different differentiation states of the muscles used.

As suggested for various ion channels gated by voltage (e.g., Ref. 7) or ligand binding (e.g., Ref. 21), membrane stretch can only be considered a modulating factor in the case of skeletal muscle K\(_{\text{Ca}}\) channels, since stretch-induced potentiation could only be revealed in the presence of an activating concentration of Ca\(^{2+}\) at the cytoplasmic face.

Activation of K\(_{\text{Ca}}\) channels in response to membrane stretch and not mediated by an increase in Ca\(^{2+}\) entry has also been observed in osteoblast-like cells, renal cells, vascular smooth muscle cells, and neurons (5, 13, 17, 19). In these preparations, channel activation was observed in the same range of pressure as in skeletal muscle cells, i.e., half-maximal activation was achieved at pressures near \(-4\) kPa. Additionally, in vascular smooth muscle cells, it was shown that stretch increased channel activity apparently without altering the voltage sensitivity of the channel (13). In our preparation, at any given potential and intracellular [Ca\(^{2+}\)], channel activity was systematically enhanced, but neither Ca\(^{2+}\) sensitivity nor voltage sensitivity of the K\(_{\text{Ca}}\) channel was significantly altered by membrane stretch. It is thus likely that stretch activation of skeletal muscle K\(_{\text{Ca}}\) channels involves neither the voltage sensor portion nor the Ca\(^{2+}\) sensor portion of the K\(_{\text{Ca}}\) channel.

We showed that an increase in K\(_{\text{Ca}}\) channel activity could also be induced by membrane stretch in cell-attached patches under high external [Ca\(^{2+}\)] conditions and large depolarizations. Although we cannot exclude the possibility that the suspected high submembranous [Ca\(^{2+}\)] might have some influence on channel properties, this suggests that the K\(_{\text{Ca}}\) channel is stretch sensitive in the presence of the intracellular environment and does not result, in excised patches, from some alteration of subsarcolemmal components subsequent to patch excision.

![Fig. 5. Effect of application of negative pressure on K\(_{\text{Ca}}\) channel activity in a cell-attached patch. Patch potential was +40 mV. Bath and intrapipette solutions contained 50 mM Ca\(^{2+}\). Bars indicate periods during which negative pressure was applied.](http://ajpcell.physiology.org/content/35/4/C477/F5)

This content is from the paper "Stretch Activation of Skeletal Muscle K\(_{\text{Ca}}\) Channels" published in *American Journal of Physiology-Cell Physiology* by Frank Schneeberger and colleagues. The figure and the text in the paper provide insights into the role of membrane stretch in activating K\(_{\text{Ca}}\) channels in skeletal muscle fibers. The authors demonstrate that suction of the membrane through the patch pipette increases the activity of K\(_{\text{Ca}}\) channels, and this effect is reversible. They suggest that this activation is not mediated by an increase in intracellular Ca\(^{2+}\) and point to the likelihood that stretch activates the channel by altering channel properties, possibly involving changes in the channel's conformation or interaction with other cellular factors.
In adult mouse skeletal muscle fibers, another mechanosensitive ion channel has been described. In cell-attached patches, Franco-Obregon and Lansman (6) observed opening of nonselective cation channels carrying inward currents at negative potentials, the activity of which was augmented by membrane stretch. We used different experimental conditions (inside-out configuration, patch potential of 0 mV) that did not enable us to observe opening of this channel even in the presence of pressure in the pipette.

Mechanosensitive ion channels are found in many mammalian cell types, in which they are thought to be involved in the regulation of cell volume and growth (9, 18, 22, 23). In skeletal muscle, although it is poorly documented, mechanosensitivity of ion channels may have physiological relevance in a contracting cell. During muscle activity, the sarcolemma is indeed thought to sustain vigorous deformations. Pardo et al. (20) proposed that costameres, vinculin-rich, submembranous transverse ribs, play a role in anchoring the Z and M lines of the outer myofilaments to the sarcolemma. In contracted skeletal muscle fibers, it was observed that the sarcolemma was thrown into bulges (or festoons) between adjacent costameres (25). Although little is known about the magnitude of tensions the membrane undergoes, membrane bulging in an active fiber may stretch the sarcolemma to the point of activation of stretch-sensitive channels.

Prolonged muscle depolarizations activate K\textsubscript{Ca} channels via voltage-activated sarcoplasmic reticulum Ca\textsuperscript{2+} release (10), but a dissociation is observed between the presumed subsarcolemmal [Ca\textsuperscript{2+}] estimated from the K\textsubscript{Ca} channel activity and the global cytosolic [Ca\textsuperscript{2+}] measured by fluorescence methods. Our findings could explain, at least partially, this discrepancy. The slight contractile response induced by the increase in cytosolic [Ca\textsuperscript{2+}] may stretch activate the K\textsubscript{Ca} channel and lead to an overestimation of the subsarcolemmal [Ca\textsuperscript{2+}].

During sustained muscle activity or in exhausted muscle fibers, the combination of slight depolarization and increased resting intracellular [Ca\textsuperscript{2+}] (11, 29) plus membrane stretch could cause K\textsubscript{Ca} channel opening. These channels may thus exert a protective effect against excessive deleterious muscle contractions by promoting a hyperpolarizing K\textsuperscript{+} outflow that forces the cell to rest.

We thank Vincent Jacquemond and Oger Rougier for helpful discussion during preparation of this paper.

This study was supported by the Centre National de la Recherche Scientifique, the Université Claude Bernard Lyon 1, and the Association Française contre les Myopathies.

Address for reprint requests and other correspondence: B. Allard, Laboratoire de Physiologie des Éléments Excitables, UMR CNRS 5578, Université C. Bernard Lyon I, 43 Boulevard du 11 Novembre 1918, Bât. 401B, 69622 Villeurbanne Cedex, France (E-mail: bruno.allard@physio.univ-lyon1.fr).

Received 28 May 1999; accepted in final form 7 October 1999.

REFERENCES
1. Allard, B., J.-C. Bernengo, O. Rougier, and V. Jacquemond. Intracellular Ca\textsuperscript{2+} changes and Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel activation induced by acetylcholine at the end-plate of mouse skeletal muscle fibers. J. Physiol. (Lond.) 494: 337–349, 1996.
10. Jacquemond, V., and B. Allard. Activation of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels by an increase in intracellular Ca\textsuperscript{2+} induced by depolarization of mouse skeletal muscle fibres. J. Physiol. (Lond.) 509: 93–102, 1998.