

## STRETCH-ACTIVATED CATION CHANNELS IN HUMAN FIBROBLASTS

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**ABSTRACT** Nonconfluent fibroblasts are relatively depolarized when compared with confluent fibroblasts, and transient hyperpolarizations result from a range of external stimuli as well as internal cellular activities. This electrical activity ceases, along with growth and mitogenic activity, when the cells become confluent. A calcium-activated potassium conductance is thought to be responsible for these hyperpolarizations, but in human fibroblasts the large calcium-activated potassium channel is not stretch-activated. We report here the identification of single stretch-activated cation channels in human fibroblasts, using the cell-attached and inside-out patch clamp techniques. The most prominent channel had a conductance of  $\sim 60$  pS (picoSiemens) in 140 mM potassium and was permeable to potassium and sodium. The channel showed significant adaptation of activity when stretch was maintained over a period of several seconds, but a static component persisted for much longer periods. Higher conductance channels were also observed in a few excised patches.

Fibroblasts respond to various external stimuli with complex changes in membrane potential. Nonconfluent fibroblasts are relatively depolarized when compared with confluent fibroblasts and they transiently hyperpolarize when subjected to mechanical, electrical, or chemical stimulation (Nelson et al., 1972). Spontaneous oscillatory hyperpolarizations are also seen and these have been associated with phagocytosis, pinocytosis and movement (Okada et al., 1981; Oliveira-Castro, 1983; Tsuchiya et al., 1981). These electrical disturbances cease, along with growth and mitogenic activity, when the cells become confluent (Binggeli and Weinstein, 1986). A calcium-activated potassium conductance has been linked to these hyperpolarizations (Hosoi and Slayman, 1985; Okada et al., 1986) but we have previously reported that the large calcium-activated channel in these cells is not stretch-activated (French and Stockbridge, 1988). No other direct mechanism for transduction of mechanical stimuli has yet been discovered.

Here, stretch-activated channels were found on the borders of human skin fibroblasts in nonconfluent tissue culture. The cells were from a maintained line of ATCC #CCL 110 (French and Stockbridge, 1988). Cells were plated at low density and were not trypsinized so that their normal nonconfluent morphology was retained. For all experiments, the pipette solution contained (mM): KCl 140,  $\text{MgCl}_2$  2, EGTA 11 and Hepes 10, pH = 7.2–7.4. The bath solution contained Hepes-buffered saline (mM):

NaCl 150, KCl 5,  $\text{CaCl}_2$  2,  $\text{MgCl}_2$  1, Hepes 10 (pH = 7.2). Temperature was maintained at  $35 \pm 2^\circ\text{C}$ . Single-channel recordings were made with a List-Electronic EPC-7 amplifier. The data were filtered at 10 kHz with a 3-pole Bessel filter, digitized, and stored on video tape. Data were analyzed on a digital computer after sampling at 20 KHz and could be further filtered at 1–5 kHz with a Gaussian digital filter. The stretch stimulus was delivered as suction to the back of the patch pipette with a 1 cc syringe and was measured with a calibrated transducer within the range of 0–70 mm Hg.

The procedures which were used to measure the amplitudes and durations of channel openings and closings have been described in detail previously (French and Stockbridge, 1988). These were based closely on the procedures described by Colquhoun and Sigworth (1983) using a half amplitude criterion to distinguish openings and closings. Final estimates of channel amplitude were obtained from the mean of the distribution of amplitudes during all openings longer than three times the filter risetime. Channel conductance and ionic selectivity were determined by fitting the Goldman-Hodgkin-Katz current equations (Hille, 1984) to the current amplitudes at a range of holding voltages using a minimum square error procedure. For cell-attached patches, an intracellular potassium concentration of 140 mM was assumed and the data were fitted by linear regression. The resultant linear plot was normalized to pass through the current-voltage intercept, allowing easier comparison with excised patch data and yielding estimates of cell membrane potentials.

For kinetic analysis, histograms of open-time and

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closed-time probabilities versus duration were created using a variable bin width algorithm to ensure a minimum of five events in any bin (Guharay and Sachs, 1984). Histograms were corrected for missed short events due to the combination of filter risetime and discriminator threshold level, and then multiple exponential probability densities were fitted to the histogram bin areas using the chi-squared technique (Colquhoun and Sigworth, 1983).

Stretch-activated channels (Fig. 1 *a*) were easily distinguished from other channels. They were silent in the absence of a stretch stimulus but responded reliably to a suction of 20 mmHg, which is less than the suction required to form a patch. The channels were almost entirely limited to the cell borders. This regional distribution of particular channels has been found with other preparations (Brew et al., 1986; Newman, 1986). Most patches contained 0, 1, or 2 channels, although up to 18 were seen in individual patches. The probability of obtaining a channel in a patch was low ( $P = 0.25$  in 102 patches), suggesting that channels are clustered. Patches on fibroblasts are relatively unstable and often contain other, larger, potassium channels (French and Stock-

bridge, 1988). Although stretch-activated channels were clearly identified in 25 patches, the present results were obtained from 4 patches in which channels were seen alone and for sufficient time to allow characterization of conductance and stretch sensitivity.

Single-channel recordings of a stretch-activated channel are shown in Fig. 1 *a* and current-voltage relationships for 4 stretch-activated channels are shown in Fig. 1 *b*. There was no spontaneous activity at rest, but suction on the pipette as low as 10 mmHg could open the channel. Suction had no effect on channel conductance which was  $58 \pm 3$  pS in 140 mM potassium. The mean relative  $\text{Na}^+/\text{K}^+$  permeability was 0.53, but was as high as 0.8 in one channel. Spontaneous membrane potential oscillations (10–30 mV) may be responsible for the curvature of the cell-attached data, since this did not occur in excised patches.

Kinetic analysis of stationary channel activity indicated multiple open and closed states (Colquhoun and Hawkes, 1981). At least two exponentials, both with relatively short time constants, were required to fit the open time histogram (Fig. 1 *c*). The closed time histogram could be fitted

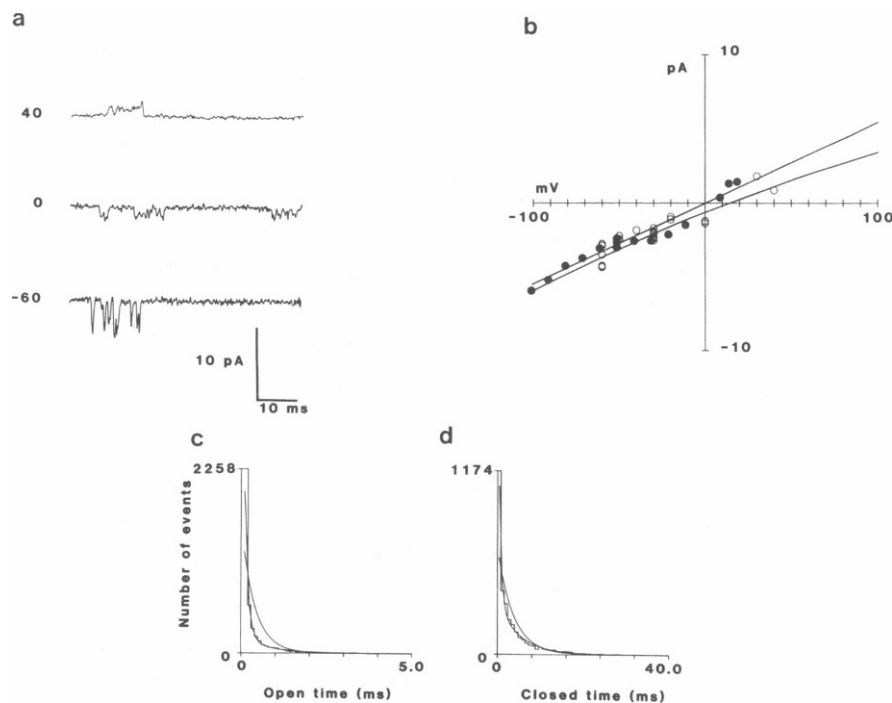


FIGURE 1 (*a*) Single-channel recordings from an inside-out patch containing the stretch-activated channel held at 40 mV (*top*), 0 mV (*middle*), and -60 mV (*bottom*), during steps to 60 mm Hg suction. Data were filtered at 2 kHz. Inward current is shown downwards. (*b*) Current-voltage plots from a cell-attached patch (*closed circles*) and three inside-out patches in Hepes buffered saline (*open circles*). Cell-attached data were fitted by a linear plot of 58.6 pS normalized to pass through the current-voltage intercept (*upper line*). Inside-out patch data were fitted with a Goldman-Hodgkin-Katz current equation of 54.6 pS and relative  $\text{Na}^+/\text{K}^+$  permeability ratio of 0.53 (*lower line*). (*c, d*) Histograms of open and closed times for a cell-attached patch held at 60 mmHg and resting membrane potential (holding potential - 0 mV). Lines show fits with single and double exponential functions. In both cases double exponentials fitted better than single exponentials by eye and by the minimum chi-squared error criterion. The open time distribution, was best fitted with time constants of 0.11 and 0.8 ms. The closed time distribution was best fitted by time constants of 0.5 and 5.1 ms.

with two exponentials during the long bursts that accompanied suction to higher pressures (Fig. 1 *d*). However, a third exponential was required to account for longer closed intervals during periods of lower channel activity. At constant voltage, the shorter open time constant increased ~220% and the longer open time constant increased ~120% over the 0–70 mmHg range. No significant changes were seen in the closed time constants. At constant pressure, no time constants changed significantly over the range of –60 to +60 mV holding potential, indicating an absence of voltage sensitivity in the channel.

The open probability of a channel ( $P_{\text{open}}$ ) was measured by dividing the time that the channel spent in the open state by the total sample time. In general,  $P_{\text{open}}$  increased with increasing suction. The absolute relationship between  $P_{\text{open}}$  and applied pressure varied between patches and decreased with time during experiments. This variability in stretch-sensitivity has been described in other stretch-activated channels (Sigurdson et al., 1987; Christensen, 1987) but its cause is unclear. Varying pipette diameter and patch size could cause variation between patches, while changes in cytoskeletal components might contribute to variations during experiments.

Two stimulus paradigms were used: (*A*) steps made directly from 0 mmHg to a fixed suction level with a subsequent return to 0 mmHg before the next step, and (*B*) suction from 0 to 70 mmHg through successive steps of 10–20 mmHg without returning to 0 mmHg. These two paradigms gave different results (Fig. 2). There was a monotonic increase in  $P_{\text{open}}$  with suction for the first paradigm. For the second paradigm, there was a small peak around 20 mmHg and a trough at ~40 mmHg, followed by a monotonic increase with further suction. This biphasic response to serial suction was reproducible throughout each experiment, even though the maximum  $P_{\text{open}}$  generally decreased with the experimental duration.

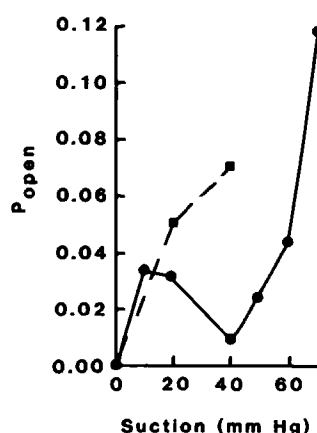


FIGURE 2 Plots indicating that the probability of the channel being open versus suction differed with the stimulus paradigm used. Direct steps from 0 mmHg to fixed pressures gave a monotonic increase in  $P_{\text{open}}$  (squares, broken line). This plot was from a cell-attached patch but similar results were seen with excised patches. Suction applied in successive steps from 0 to 70 mmHg gave a more complex results (circles, solid line). Similar results were obtained in five experiments on three excised patches

with minima at 40 mmHg in each case and a mean response at 60 mmHg of  $P_{\text{open}} = 0.046 \pm 0.024$ . In the cell-attached experiment a similar shape was seen but the minimum occurred at 50 mmHg. All data were obtained with 0 mV holding potential. The  $P_{\text{open}}$  values were based on the first 2.5 s of data after the change in pressure in each case.

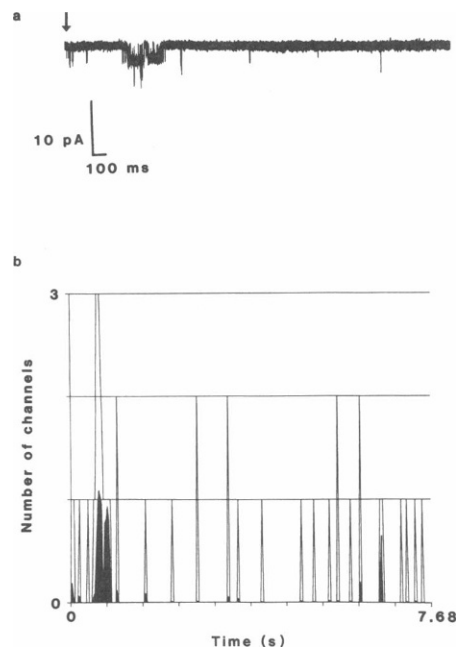


FIGURE 3 (*a*) Single-channel recording showing the first 2.5 s after the onset of suction (arrow) during a direct pull from 0 to 60 mmHg. Data were filtered at 3 kHz. There were three channels in the patch and the individual peak channel height was 2.4 pA at –30 mV. (*b*) Peak channel openings sampled every 30 ms for 7.68 s after the onset of suction. Shaded areas indicate the mean time that channels were open during the same time interval.

When  $P_{\text{open}}$  was measured as a function of time after a suction step, there was a decrease in activity within the first 5 s. Thus, the channel exhibited adaptation. To illustrate this better, Fig. 3 *a* shows the patch current obtained after a suction step to 60 mmHg. Adaptation of the initially high activity was followed by a sustained, nonadapting level of activity (Fig. 3 *b*). This sustained activity increased with increasing suction. The trough at 40 mmHg was probably caused by adaptation of initial activity, while the monotonic increase at higher suctions was due to a combination of the sustained component and activity caused by the individual suction steps. The biphasic relationship was reflected in changes of open times within bursts, with little or no effect on closed times between bursts.

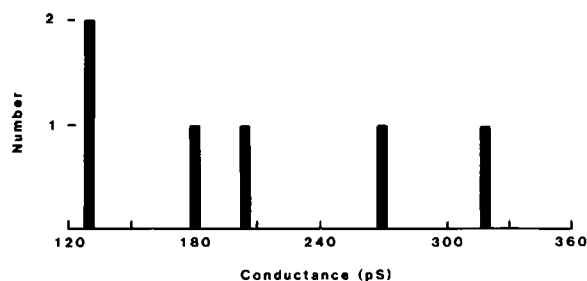


FIGURE 4 Histogram of higher conductance channels which were stretch-activated. All conductances were measured from excised patches in Hepes-buffered saline.

In a few experiments ( $n = 6$ ), stretch-activated channels of higher conductances were seen (Fig. 4). Their conductances were close to multiples of 30 and 60 pS. No indication of subconductance states could be seen in the recordings. These observations might be due to several channels opening in concert or to other channels unrelated to the 60 pS channel. Patches containing these channels were so uncommon that they were difficult to characterize.

Stretch-activated channels with similar conductances and selectivity have been reported in several preparations (Guharay and Sachs, 1984; Cooper et al., 1986; Sigurdson et al., 1987) but this channel is novel in exhibiting adaptation, as well as sustained activity, in response to suction. It also seems different to stretch-activated channels of lower conductance (Christensen, 1987; Lansman et al., 1987). It has been suggested that other stretch-activated channels are involved in the detection of mechanical stress or cell volume changes. The role of this stretch-activated channel in cell function is unclear, but its high sodium permeability suggests that channel stimulation would cause depolarization. Increases in intracellular sodium (Binggeli and Weinstein, 1986) and calcium (Okada et al., 1986) are central to two current theories describing activity and membrane potential changes in nonconfluent fibroblasts. Mechanically-activated channels permeant to sodium could, either directly or indirectly, provide the transduction mechanism required for these processes. The location of these stretch-activated channels at the cell border, where continual movements are taking place, suggests that they are likely to be involved in stress measurement due to cell movement, rather than volume regulation.

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