



A stretch-sensitive Cl⁻ channel in human corpus cavernosal myocytes

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With the patch clamp method we demonstrate a stretch-sensitive Cl⁻ currents as well as stretch-sensitive Cl⁻ channels in a small group ($\approx 5\%$, $n = 117$) of cultured human corpus cavernosal muscle cells. The current and the channel activities had the following characteristics: (1) Their equilibrium potentials changed with extracellular Cl⁻ concentration close to that predicted by Nernst equation provided that the relevant channels had high permeability to Cl⁻ but low permeability to acetate ions; (2) They were blocked by mM concentrations of Zn²⁺; (3) The *i-v* relation of single channel current was almost linear for holding potentials varied from -70 to $+60$ mV; and (4) The channels had unitary conductances of $\approx 140-170$ pS.

Keywords: corpus cavernosal myocyte; stretch-sensitive Cl⁻ channel

Introduction

The pioneering work of Boyle and Conway¹ demonstrates that the skeletal muscle cell membrane was permeable to Cl⁻. Cl⁻ is distributed almost at equilibrium, so that the equilibrium potential of Cl⁻, E_{Cl} is close to the resting membrane potential. The intracellular Cl⁻ concentration in vascular smooth muscle is almost double of that found in skeletal muscle. They are in excess of the expected levels if they are passively distributed. The high intracellular Cl⁻ concentration is maintained by active Cl⁻-HCO₃⁻ exchange and Na⁺-K⁺-Cl⁻ cotransport.² The E_{Cl} (≈ -30 mV) is much less negative than the resting membrane potential (≈ -50 mV).²⁻⁵ Electrophysiological studies showed that increase the membrane permeability to Cl⁻ by norepinephrine depolarized the membrane potential of arterial and veinal smooth muscle cells.^{3,4,6,7} Substitution of Cl⁻ with other anions affects vascular smooth muscle resting tension and vascular reactivity to some vasoconstrictor stimuli as well.^{2,8-10} Furosemide, an inhibitor of anion-cation-cotransport decreases intracellular chloride content and exerts a vasodilator effect on rabbit aorta.² These

results indicate that Cl⁻ is involved in the regulation of vascular smooth muscle tone.

Clinical as well as experimental results indicate that the level of tone of human corpus cavernosal smooth muscle cells (cavernosal myocytes) is closely linked to the erectile function.¹¹⁻¹⁴ Based on the obvious similarities in the structural and pharmacological properties between cavernosal myocytes and vascular smooth muscle cells,^{12,15-18} Cl⁻ might be expected also to play a role in the regulation of tone of cavernosal myocytes and, thus, to the erectile function.

In this work we report the putative stretch-sensitive Cl⁻ channels in cultured human corpus cavernosum smooth muscle cells.

Materials and methods

Cell cultures

All studies were performed according to a protocol approved by the Internal Review Board of the Albert Einstein College of Medicine/Montefiore Medical Center. Human erectile tissue was obtained from the corpora cavernosum of one patient for removal of penile carcinoma and five patients undergoing surgery for implantation of penile prostheses. Methods of cell culture can be found in Campos de Carvalho *et al.*¹⁹ Cells of passages 1-4 were used.

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Solutions used

For whole-cell and cell-attached patch recording bath solution contained (in mM) 140 NaCl, 0.1 CaCl₂, 0.6 EGTA and 10 HEPES, pH 7.2 (NaCl solution). Pipette solution for whole cell recording contained (in mM) 140 KCl, 0.1 CaCl₂, 0.6 EGTA 1 MgCl₂, 2 K-ATP and 10 HEPES, pH 7.2. NaCl solution was used as the pipette solution for cell-attached patch recording.

Electrophysiological methods and analysis of the single channel current data

The conventional tight-seal method²⁰ was used. Details can be found in our previous works.^{21,22}

All drugs used were from Sigma Chemical Company (St. Louis, MO). Experiments were performed at room temperature (22–24°C).

Results

Whole-cell Cl⁻ current

Characteristics of Cl⁻ current. We recorded whole-cell currents from 68 cells bathed in NaCl solution during pulsed depolarization and pulsed

hyperpolarization. Among them, 2 cells showed inward currents composed of a transient and a long lasting component during the application of hyperpolarizing step bigger than -60 mV (Figure 1A). The transient component decayed with a time constant of about 2 msec. It was blocked by Cd²⁺ (1–2 mM) and vanished after the extracellular Ca²⁺ concentration ([Ca²⁺]_o) was raised to 2 × 10⁻⁶ M indicating that it represents the monovalent cations in the bath solution passing through T-type Ca²⁺ channels^{23,24} and was not carried by Cl⁻. With a 2 sec duration depolarization pulse the long lasting component had a decay constant > 0.5 sec. It was sensitive to neither to Cd²⁺ nor to extracellular Ca²⁺ concentration but was eliminated by the presence of 2 mM ZnCl₂ (Figure 1B). Extrapolating the linear portion of the I–V curve revealed the zero current points which approximate membrane potentials of -33 and -29 mV for the two cells, respectively. In one cell (the one with equilibrium potential equals to -33 mV) we changed the Cl⁻ concentration of the bath solution by substituting part of the NaCl with equimolar sodium acetate. The equilibrium potentials shift to -18 and -1.5 mV as 50 and 75% of NaCl were substituted, respectively (Figure 1C). As judged from the change of equilibrium potential with the composition of anions in bath solution, the relevant channels would have low permeability to both cations and acetate ions but high permeability to Cl⁻, that is, they were Cl⁻ channels.

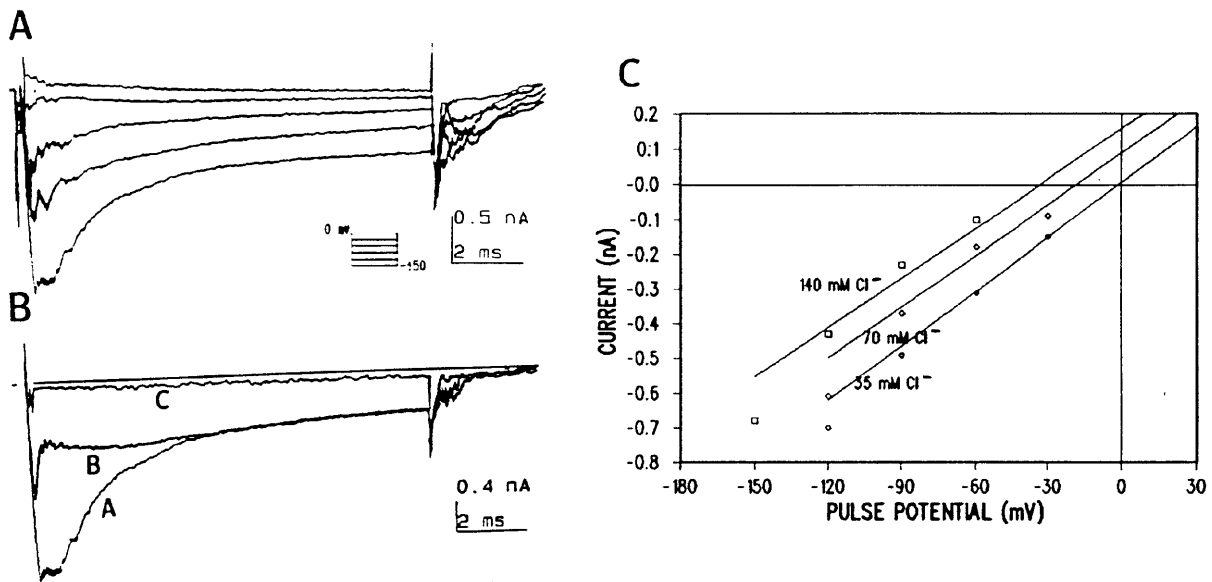


Figure 1 Inward putative chloride current. (A) Whole-cell inward Cl⁻ current induced by voltage pulses from -30 to -150 mV in five steps. Holding potential was 0 mV. (B) Effect of 2 mM CdCl₂ and 2 mM ZnCl₂ on inward current. Curve A. Control. Current elicited by a -150 mV pulse. Curve B. With the addition of 2 mM CdCl₂ into the bath solution. Curve C. With further addition of 2 mM ZnCl₂ into the bath solution. Holding potential was 0 mV. (C) I-V relation of cell bathed in solution with 140, 70 and 35 mM Cl⁻ substituted with acetate ions respectively.

Effect of stretching of the cell surface membrane

In many cell types as the cell membrane is stretched, such as that happened during hyposmotic shock, Cl⁻ channels are activated.²⁵⁻³⁰ To test whether the putative Cl⁻ conductance of cavernosal myocytes is sensitive to stretch, we applied positive pressure (about 15 mm H₂O) intracellularly through the recording pipette. Among 30 cells that did not show signs of Cl⁻ current 2 showed inward Cl⁻ current during high hyperpolarizing pulses with the application of positive pressure. The inward current developed (Figures 2A-A and 2A-B were records taken before and after applying pressure) was similar to that shown in Figure 1B. It was not sensitive to extracellular Cd²⁺ (2 mM) (Figure 2A-C) but sensitive to extracellular Zn²⁺ (2 mM) (Figure 2A-D). The amplitude of the inward current was holding potential dependent, decreased as the holding potentials became less negative and vanished as the holding potential was higher than about +50 mV (Figure 2B). Their equilibrium potentials were -31 and -35 mV, respectively. Since cell lost readily when the pressure applied was increased, we were unable to get data concerning the pressure vs Cl⁻ conductance of the cell.

Cl⁻ channel activities and the effect of stretching in cell-attached patches

In 49 cell-attached patches tested two of them showed Cl⁻ channel activity at high positive holding potential. We then applied negative pressure (about 15 mm H₂O) through the recording pipette. Among the 22 successful experiments, those that did not show Cl⁻ channel activity before applying negative pressure, remained so. For the two that originally had Cl⁻ channel activities, the open probability increased. The increase of channel open probability in the patch from one cell was reversible for the first application of negative pressure but persisted after the second application. In a patch from another cell, increase of open probability persisted after the first application of negative pressure (Figure 3A). Figure 3B was the record of the channel activity of the first cell at a holding potential of -70 mV. In Figures 3B-b, B-d and B-e, several levels of opening can be seen. Those with unitary currents of 7.1 and 3.2 pA were most prominent. They had open probabilities of 0.048 and 0.023, respectively. The latter was probably the substate openings of the channel since shift of current between these two levels could be seen. Occasionally, openings with unitary current of 11 pA appeared. We are not sure whether they were openings of another type of channel or were the results of simultaneously opening of more than one channel. Their equilibrium potentials were around -30 mV indicating that they all were Cl⁻ channels. Figure 3C shows the i-v curves of channels in the two patches with highest open probabilities. Their unitary conductances were 164 and 138 pS, respectively. Figure 3D shows the open probabilities vs holding potential relationship of the 164 and the 138 pS channels. For the 164 pS channel, the curve was bell-shaped. Its open probability was very low for holding potentials from about -30 to +10 mV. The other one only opened occasionally at holding potentials higher than -30 mV.

In a patch from another cell, after applying negative pressure, more than one channel opening with different open and closed kinetics appeared. Figure 4A-a was taken during applying negative pressure and 4A-b and 4A-c were taken after releasing negative pressure. All show similar features. Both histograms of closed and open time taken during or after applying negative pressure could be fitted with two exponentials. Figure 4B shows histograms of 1 min data taken during the application of negative pressure. Their time constants were 25.5, 0.57 and 4.39, 0.48 msec for closed- and open-time respectively. The channel with longer open time always opens in bursts. Figure 4A was taken during the burst, which might give a wrong impression that their open probability was quite

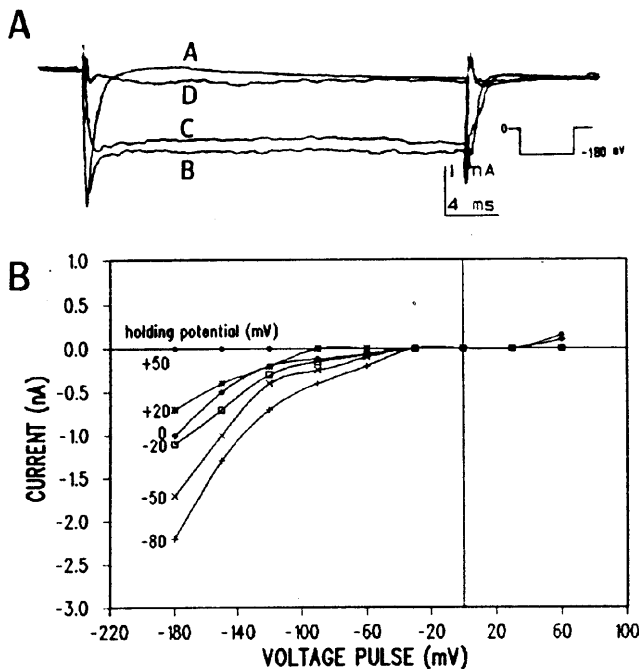


Figure 2 Effect of stretch (≈ 15 mm H₂O positive pressure applied through the recording pipette) on the putative Cl⁻ current. (A) Whole-cell inward current elicited by a -180 mV pulse. Curve A. Control. Curve B. After applying pressure. Curve C. With the addition of 2 mM CdCl₂ into the bath solution. Curve D. With further addition of 2 mM CdCl₂ into the bath solution. Holding potential was 0 mV. (B) I-V relations at different holding potentials after applying pressure.

high. The corresponding time constants taken after applying negative pressure were 29.8, 0.73 and 4.13, 0.57 msec, respectively.

Cl⁻ channel activity in inside-out cell-detached patches

Large conductance Ca²⁺ sensitive Cl⁻ channels (unitary conductance > 300 pS) have been reported in vascular smooth muscles.³¹ As in cases of other cell types (for example type II alveolar cell,³² macrophage,³³ skeletal muscle³³⁻³⁵) open probability of channels from vascular smooth muscle cells are very low in cell-attached patches. They will

usually open after forming inside-out cell-detached patches. To test whether the stretch-sensitive Cl⁻ channel observed in cavernosal myocytes resembled this type, we performed single channel recordings in inside-out cell-detached patches from 28 cells. We obtained openings of channel with unitary conductance equals to 323 pS from one patch. Its equilibrium potential changes with Cl⁻ concentration in the bath solution close to that predicted by Nernst equation if it is a Cl⁻ channel. Its open probability was sensitive to Ca²⁺ concentration of bath solution (namely at the intracellular side). However, the open probability did not change as a negative pressure was applied. Therefore the stretch-sensitive channel demonstrated belongs to a different category.

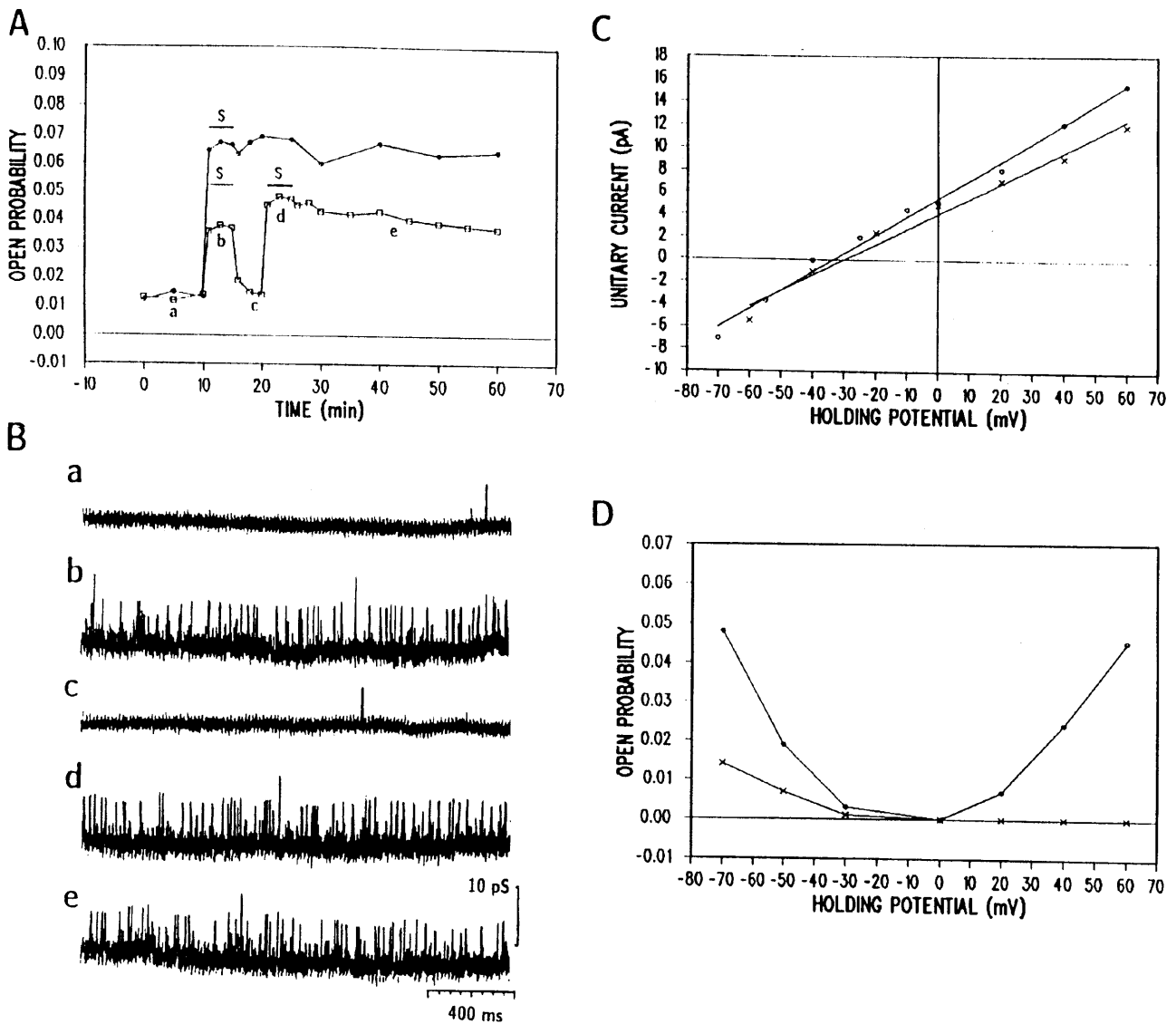


Figure 3 Effect of stretch (≈ 15 mm H₂O negative pressure applied through the recording pipette) on the activity of the putative Cl⁻ channel. (A) Change of open probability with suction (S) of two cell-attached patches from two cells. Holding potential was -70 mV. (B) Current records from one patch. a, b, c, d and e corresponding to the time of recording labeled in A. (C) i-v relations of two channels with the highest open probabilities from two cells respectively. (D) Open probability-holding potential relation of the two channels.

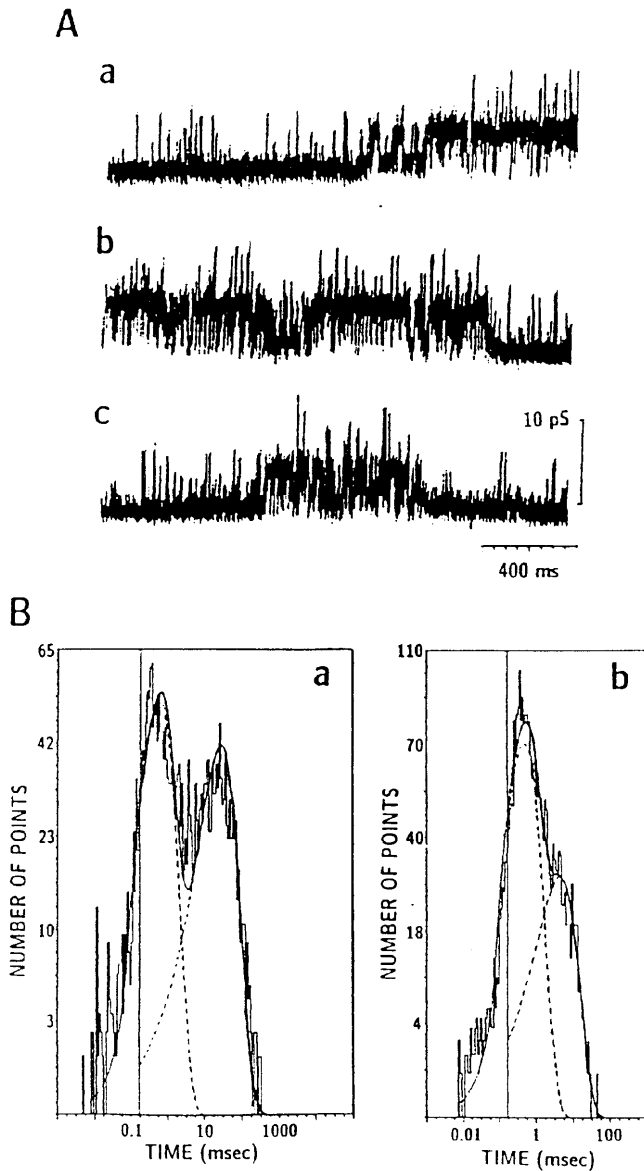


Figure 4 Channel openings with different activation and inactivation kinetics after suction. Records taken from a cell-attached patch different from that for obtaining record shown in Figure 3. Holding potential was -60 mV. (A) Current record a taken during suction, records b and c taken after the negative pressure was released. (B) Closed-time (a) and open-time histograms. Both can be fitted with two exponentials. Ordinate: Number of points in square root scale. Abscissa: Time in log scale. Sampling time 100 μ sec. Vertical line close to 0.1 msec denotes the dead time of the recording system.

Discussion

The stretch-sensitive conductance is a Cl⁻ conductance

As judged from the following facts, the stretch-sensitive conductance documented in this work is a

Cl⁻ conductance: (1) Its equilibrium potential (≈ -30 mV) was about the same as that reported for the Cl⁻ equilibrium potential of vascular smooth muscle cells (-12 to -40 mV² and ≈ -30 mV)³⁶ and shifted with Cl⁻ concentration (substituted with equimolar acetate ions) of the extracellular medium close to that predicted by Nernst equation provided if the relevant channels were permeable to Cl⁻ but practically impermeable to acetate ions; (2) It was sensitive to Zn²⁺. Zn²⁺ is known to block Cl⁻ channels;^{37,38} and (3) With the assumption that the observed channels are Cl⁻ channels, the intracellular free Cl⁻ concentrations deduced from the equilibrium potential were 35 and 46 mM for the two cells, respectively. These values are in consistent with that reported for smooth muscle measured by various techniques (around 40 mM).³⁹

The tip diameters of the pipette we used were about $1-2$ μ m and the pipettes were filled with a solution containing 140 mM Cl⁻. If the cell has no Cl⁻ homeostasis system, the intracellular Cl⁻ concentration ($[Cl^-]_i$) would increase and reach a steady state with a time constant of $2-5$ min.⁴⁰ The reversal potential was close to zero at an extracellular concentration of 35 mM indicating that the $[Cl^-]_i$ was well controlled by the Cl⁻ transport systems.²⁻⁵ Similar results were reported for guinea-pig vas deferens.³⁹ The $[Cl^-]_i$ of vas deferens is also substantially higher than that predicted from a passive distribution with E_{Cl} over 40 mV positive to the normal membrane potential.

Stretch-sensitive Cl⁻ channels of various cells so far reported could be divided into two categories: one with unitary conductance smaller than 10 pS (for example, ~ 9 pS in cell line T84,²⁹ ≈ 2 pS in choroid plexus epithelium,⁴¹ ≈ 7 pS in Ehrlich ascrites tumor cells⁴²) and one outward-rectifying with outward slope unitary conductance around $50-60$ pS (for example, ≈ 50 pS in epithelial cells,²⁷ ≈ 63 pS in Madin Darby Canine Kidney cells²⁵). The stretch-sensitive channels reported in this work could not be classified into either one. Not only the unitary conductances were different, but they were not outward-rectifying. They were not the substate of the big Ca²⁺-activated Cl⁻ channel, since the big channels in corpus cavernosal myocytes was not sensitive to stretch of the membrane. It seems that these Cl⁻ channels are a new subtype of stretch-sensitive Cl⁻ channel. Shoemaker *et al*⁴³ reported Cl⁻ channel with unitary conductance > 150 pS in cultured rat carotid smooth muscle cells.

On the mechanism of stretch activation of Cl⁻ channel

Two mechanisms have been proposed for the activation of Cl⁻ channels by stretching. Firstly, as a direct consequence of stretch such that stretching

of the membrane changes the conformation of the channel protein molecules. The secondary results from the activation of intracellular signal pathways via increase of intracellular Ca²⁺ concentration.^{44,45} The activity of big Ca²⁺-activated K⁺ channel (maxi-K⁺ channel) did not show increase by stretching rendering the later option improbable. However, other intracellular signal pathways such as that involving a G protein and arachidonic acid metabolites as suggested by Doroshenko and Nehr,⁴⁶ were not excluded.

On the possible functional significance

Water is in thermodynamic equilibrium across the surface membrane of animal cells. Because cytoplasm contains a higher concentration of impermeants, a driving force exists that pull small ions and water into the cell. Since the cell membrane is fragile and cannot sustain a hydrostatic pressure gradient, a mechanism which can oppose the tendency of cells to swell is necessary, stretch sensitive Cl⁻ channels play an important role. Muscle cells, however, encounter stretch from another origin—stretch due to passive lengthening of the cell by force external to the cell. A series of events could be initiated: activation of the stretch-sensitive Cl⁻ channel, depolarization of the surface membrane, increasing of Ca²⁺ influx then activation of the contractile apparatus to counteract the external force. In this way, stretch-sensitive Cl⁻ channel could involve in the servo-mechanism of length maintenance of muscle cell.

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