Two Types of Stretch-Activated Channel Activities in Guinea-Pig Gastric Smooth Muscle Cells

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Abstract: Using cell-attached patch-clamp techniques, stretch-activated channels were investigated in guinea-pig gastric smooth muscle cells. Suction applied to the pipette interior or cell swelling induced by hypotonic bath solution induced two kinds of channel openings, a short-lasting and small opening (O_5) and a long-lasting and large opening (O_6) with conductances of 33 and 53 pS, respectively. Threshold suction for O_5 was lower than that for O_6. In many patches examined, O_5 and O_6 coexisted. The distributions of open- and closed-times of O_5 could be fitted by the sum of two exponentials, the time constants of which were 1.3 and 9.3 ms for the open-time, and 0.6 and 8.4 ms for the closed-time. Both O_5 and O_6 were blocked by 100 μM Gd^{3+} in the pipette. The reversal potentials did not change upon altering [Cl^-]. The results indicate that O_5 and O_6 seem to represent non-selective cation channel activities, which do not discriminate among K^+, Na^+, and Ca^{2+}. These channels may play an important role in the stretch-induced contraction in gastric muscle. [Japanese Journal of Physiology, 46, 337–345, 1996]

Key words: stretch-activated channel, non-selective cation channel, gastric smooth muscle, patch clamp.

The stomach has sophisticated motor functions which allow food to remain until it is well mixed with the gastric juice and forms a semifluid mixture called chyme. Although neural and humoral mechanisms play major roles in controlling gastric motility, stretch of the gastric wall caused by the contents may directly affect the smooth muscle cells to cause stretch-induced contraction [1–3]. This auto-regulation could provide a basic mechanism in controlling the motility of the stomach.

After the first description by Guharay and Sachs [4], stretch-activated channels (SA channels) have been demonstrated in many kinds of cells including smooth muscle cells (in toad stomach [5], in pig coronary artery [6], and in guinea-pig urinary bladder [7]). Most of the SA channels found are Ca^{2+}-permeable, non-selective cation channels, the activation of which introduces some Ca^{2+} into the cell. This Ca^{2+} influx via SA channels activates phospholipase C [8], and stored Ca^{2+} is released by inositol 1,4,5-trisphosphate. On the other hand, the activation of SA channels depolarizes the membrane [9] and increases spike frequency, introducing more Ca^{2+} into the cell. All these mechanisms may be involved in the stretch-induced increase in the intracellular Ca^{2+} concentration [10–12], and the SA channel is thought to play a key role in the initiation of stretch-induced contraction [5].

Besides the amphibian stomach, mammalian gastro-intestinal smooth muscle cells also ought to have SA channels. The aim of the present study was to identify and characterize SA channels in mammalian gastric smooth muscle cells. The results obtained indicate that the smooth muscle cells of the guinea-pig stomach indeed exhibit two types of SA channel activities. A preliminary account of part of this study has been reported elsewhere in abstract form [13, 14].

METHODS

Cell isolation. Male guinea-pigs, weighing 300–400 g, were sacrificed by dislocation of the neck followed by exsanguination. The pyloric region of the stomach was excised and soaked in a nominally Ca^{2+}-free physiological salt solution (0-Ca PSS: compositi
tion in mm; NaCl, 145; KCl, 5.4; MgCl₂, 1; HEPES, 10; glucose, 10) at room temperature. The mucus layer was removed and the circular muscle was dissected out in strips, under a binocular microscope. After washing the strips with fresh 0-Ca PSS, they were stored at 4°C. The strips were then transferred to a 0-Ca PSS containing 0.05% (wt/vol) papain (P-4762, Sigma Chemical Company, St. Louis, MO, U.S.A.), 0.05% purified collagenase (Amano Pharmaceutical Co., Nagoya, Japan), 0.1% bovine serum albumin (A-6003, Sigma), and 1mM diithiothreitol (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and were incubated for 3h at 4°C. The temperature was then raised to 35°C for 15 min, after which the strips were rinsed with “KB medium” [15] at 4°C, and triturated with a wide-bore pipette in the same solution. Single cells were collected through nylon mesh and stored in KB medium at 4°C.

**Electrophysiological techniques.** The tight-seal patch-clamp method was used in cell-attached configuration [16]. Smooth muscle cells in the experimental chamber were continuously superfused with oxygenated bath solution, the temperature of which was kept constant at 25°C. Unless otherwise stated, high concentration potassium solution was always used in the bath to obtain zero membrane potential. Electrodes were made from Kimax glass tubing (1.5 mm o.d.) and pulled to internal tip diameters of 1–2 μm using a vertical puller (PP-83, Narishige Scientific Instruments, Tokyo, Japan). The electrode was positioned using a micro-manipulator (MX-1, Narishige Scientific Instruments). Current signals were acquired using an EPC-7 patch-clamp system (List-Medical, Darmstadt, Germany) and stored on a DAT tape with a band-width of DC-10kHz (PC-204, Sony Magnescale Inc., Tokyo, Japan). The tape was played back after each experiment, and the signal was filtered with a 4-pole low-pass Bessel filter (E-3201A, NF Circuit Design Block, Yokohama, Japan). It was then digitized with a Digidata 1200 A-D converter interfaced to a microcomputer running the pCLAMP program (Axon Instruments Inc., Foster City, CA, U.S.A.). When the patch potential was clamped with ramp-pulses, the current signals were digitized directly without storing on the tape. The sampling frequency was always five times higher than the cut-off frequency (~3 dB) of the filter (f).

Calibrated pressure for the suction was induced with a water manometer and applied to the pipette input through an electromagnetic valve. In most experiments, the duration of the suction pulse was either 5 or 10 s, applied every 10 or 20 s, respectively. In all current traces shown in the figures, downward deflections from the baseline represent inward currents. The channel activity is expressed as N̅P̅, the product of the number of active channels in the patch (N) and the open-state probability (P). Results are expressed as mean±SD, unless otherwise stated.

**Solutions.** The solutions used are listed in Table 1. The osmolality was measured using the freezing-point method (OM-6020, Kyoto Daiechi Kagaku Co., Ltd., Kyoto, Japan). The liquid junction potentials

<table>
<thead>
<tr>
<th>Table 1. Composition of solutions (mM).</th>
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<tr>
<td><strong>Solution</strong></td>
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<tr>
<td><strong>A</strong></td>
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<td>--------</td>
</tr>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>KCl</td>
</tr>
<tr>
<td>CaCl₂</td>
</tr>
<tr>
<td>MgCl₂</td>
</tr>
<tr>
<td>Potassium aspartate</td>
</tr>
<tr>
<td>EGTA</td>
</tr>
<tr>
<td>HEPES</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Sucrose</td>
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<tr>
<td>NaOH</td>
</tr>
<tr>
<td>KOH</td>
</tr>
<tr>
<td>TFA-OH</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg H₂O)</td>
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<tr>
<td>Vᵢ (mV)</td>
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</table>

The pH was adjusted to 7.3 by adding a base indicated by "+". EGTA, ethylene glycol bis (β-aminoethyl ether) N,N,N',N'-tetraacetic acid; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; TFA-OH, tetraethylammonium hydroxide. Vᵢ is the liquid junction potential. The values are presented with respect to solution A.
were measured, assuming the junction potentials at the tip of a 3 M-KCl electrode in these solutions to be negligible [17].

RESULTS

Two types of gastric SA channel activities

When cell-attached patch recording was commenced at a negative potential, two different types of channel activities spontaneously appeared immediately after gigaseal formation. One exhibited repetitive brief openings while the other exhibited longer openings with larger unitary current amplitude. The spontaneous activities usually disappeared within several minutes after seal formation. These activities, then, could be induced by application of suction to the pipette interior. Figure 1a-a shows channel activities induced by various intensities of suction. In this particular patch, short-lasting and small openings (O₂) alone were induced. Pressure-dependency of channel activation was demonstrated when the \( N \rho_0 \) was plotted against the suction intensity (Fig. 1A-b). In the other patch shown in Fig. 1B-a, O₅ alone was induced by suction of up to 10 cm H₂O. In addition to O₅, long-lasting and large openings (O₆) were induced by increasing intensity of suction. Pressure-dependency

![Graph of Nρ₀ vs. Applied suction (cm H₂O)]

**Fig. 1.** Channel activities induced by various intensities of suction. A-a, B-a: Current traces recorded from two cell-attached patches held at −50 (A) or −60 mV (B) using bath solution A and pipette solution D. The intensity of suction applied is indicated on the left of each trace and application period (10 s for A and 5 s for B) at the top. \( t_s \) was 100 Hz. A-b, B-b: Suction was repeated and the averaged \( N \rho_0 \) of O₅ (a) or O₆ (b) was obtained and plotted against the suction intensity. For the calculation of O₅, O₆ was ignored.

![Graph of Number of Observations vs. Amplitude (pA)]

**Fig. 2.** Comparison of two types of channel activities (O₅ and O₆). Current traces (a) and all-point histograms of current amplitude (b) recorded during suction of 15 (A) or 30 cm H₂O (B) in a cell-attached patch held at −60 mV using bath solution A and pipette solution D; \( t_s \) was 1 kHz. Application periods of suction are indicated at the top. Interruption in the trace in A-a is 420 ms. Histograms were fitted to Gaussian distributions (smooth curves). A and B were taken from the same patch.
of channel activation was also demonstrated for \( O_L \) (Fig. 1B-b). Thus, these channels could be called SA channels. As the SA channel activity disappeared within several minutes after the patch was excised, all recordings in the present study were taken in the cell-attached configuration. In 463 patches examined (one patch per cell), both \( O_S \) and \( O_L \) were observed in 246 patches and \( O_S \) alone was observed in 127 patches. There were no patches showing \( O_L \) alone. The threshold for \( O_L \) was always higher than that for \( O_S \), while the absolute values of these thresholds were variable among patches, probably because the dimension and the shape of the pipette tip were variable. Similar pressure-dependency as shown in Fig. 1 were observed in 6 further patches having both \( O_S \) and \( O_L \) and 7 more patches showing only \( O_S \).

The two types of openings are compared more precisely in Fig. 2. In this particular patch, suction of 15 cm \( H_2O \) induced at least 2 channels showing \( O_S \) (Fig. 2A), while suction of 30 cm \( H_2O \) induced at least 3 channels showing \( O_L \) (Fig. 2B). \( O_S \) was characterized by short open-times and relatively small unitary current amplitudes. \( O_L \) was characterized by long open-times, noisy open states, and relatively large unitary current amplitudes.

An analysis of open- and closed-times of \( O_S \) was

![Fig. 3. Channel kinetics in \( O_S \). A: Current record obtained in a cell-attached patch held at \(-80 mV\) using bath solution A and pipette solution D; \( f = 100 Hz \). Application periods of suction (20 cm \( H_2O \)) are indicated at the top. Each interruption in the trace is 3 s. From the record shown in A, distributions of open-time (B) and closed-time (C) were calculated. \( f_c \) was 1 kHz. Each histogram was fitted by the sum of two exponentials (smooth curves).](image)

![Fig. 4. Channel activities induced by a long application of suction. A-a: Current record obtained in a cell-attached patch held at \(-60 mV\) using bath solution A and pipette solution D. Suction of 18 cm \( H_2O \) was applied throughout the recording; \( f = 100 Hz \). A-b: Current record on an expanded time-scale from the indicated portion (bar) of the trace in A-a; \( f_c \) was 1 kHz. From the record shown in A, distributions of open-time (B) and closed-time (C) for \( O_L \) were obtained; \( f_c \) was 1 kHz.](image)
done on the patch which appeared to contain only one channel showing O₅ (Fig. 3). Both distributions could be fitted by the sum of two exponentials, the time constants of which were 1.3 and 9.3 ms for the open-time, and 0.6 and 8.4 ms for the closed-time (Fig. 3B, C).

In the experiment shown in Fig. 4, both O₅ and O₆ were induced by a suction of 18 cm H₂O applied throughout the recording (Fig. 4A-a). The recording on an expanded time-scale revealed the short and repetitive nature of O₅ and the long duration of O₆ (Fig. 4A-b). Figure 4B and C show the distributions of open- and closed-times of O₅, respectively, in the recording shown in Fig. 4A. The open-time ranged 12.8–1,501.4 ms (average: 389.7±404.9 ms, n=37) and the closed-time ranged 10.4–5,754.0 ms (average: 910.6±1,209.8 ms, n=36). As the open- and closed-times were distributed beyond 1 s, it was necessary to use much longer recordings than that shown in this figure for further analysis of channel kinetics. Such recordings were not available, however, because whenever we tried to apply suction for more than several minutes, the channel activity disappeared. That was the reason why we did not perform the analysis of channel kinetics for O₆. The channel activity persisted for quite a long time (up to 1 h) when the suction was applied intermittently.

**Blockade of SA channels by gadolinium**

Gadolinium has been widely used as a blocker for SA channels [18], and we examined the effect of Gd³⁺ on O₅ and O₆ (Fig. 5). As Gd³⁺ acts from the outside of the channel, we first filled the electrode from the tip with solution D, and then back-filled it with solution D but now containing 100 µM GdCl₃. Current traces taken immediately after the seal formation (time 0) revealed both O₅ and O₆, with some of O₅ activity superimposed on O₆ (Fig. 5A-a). Although the time for Gd³⁺ to diffuse to the tip varied from one electrode to the other, it was likely that Gd³⁺ would normally reach the tip well within 10 min, at which time trace in Fig. 5A-b was recorded. In the presence of Gd³⁺, O₆ disappeared leaving occasional O₅ activity. Both O₅ and O₆ could usually persist for more than 10 min in the absence of Gd³⁺. Non-parametric statistics showed that the effect of Gd³⁺ on the averaged current was significant (Fig. 5B).

**I–V relationship of gastric SA channel**

Because of the long-lasting nature of O₆, the current–voltage relationship for O₆ could be obtained by applying a voltage ramp during application of suction. When solution D (i.e. high NaCl) was applied to the patch pipette, the reversal potential of O₆ was close to zero potential (Fig. 6A). In Fig. 6B, the pipette contained a high potassium and low chloride solution (solution H). Here, the reversal potential was again close to zero potential. As the reversal potential did not change upon altering [Cl⁻]o, O₆ did not seem to be re-

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*Fig. 5. Effect of gadolinium on SA channel. A: Current traces in a cell-attached patch held at −60 mV using bath solution A. The pipette was first filled from the tip with solution D by capillary action (ca. 1 mm deep), and then back-filled with solution D containing 100 µM GdCl₃. Once the pipette was prepared, the patch was formed and the trace shown in A-a was recorded immediately (time 0). Suction of 20 cm H₂O was applied as indicated at the top; t₅₀ was 100 Hz. A-b was recorded 10 min after the establishment of the gigaseal. B: Averaged currents during a total of 5 repeated applications of suction, 5 and 10 min after the seal formation normalized by the averaged currents at time 0 in each patch. Open columns indicate the mean and SEM of normalized currents from 8 patches using pipette solution without GdCl₃, and filled columns from 5 patches with 100 µM GdCl₃. *The currents with GdCl₃ were significantly different from the currents without GdCl₃ (randomizing test, p<0.01).*
Table 2. Conductance and reversal potential (mean±SD).

<table>
<thead>
<tr>
<th>Pipette solution</th>
<th>Type</th>
<th>Conductance (pS)</th>
<th>Reversal potential (mV)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>O₄₋</td>
<td>32.7±1.6</td>
<td>−3.8±1.8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>O₃₋</td>
<td>52.9±5.0</td>
<td>−1.4±1.7</td>
<td>18</td>
</tr>
<tr>
<td>H</td>
<td>O₄₋</td>
<td>46.1±1.8</td>
<td>−2.3±1.0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>O₃₋</td>
<td>69.8±6.7</td>
<td>−0.7±1.2</td>
<td>13</td>
</tr>
<tr>
<td>E</td>
<td>O₄₋</td>
<td>42.3±5.4</td>
<td>−3.7±1.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>O₃₋</td>
<td>77.8±7.5</td>
<td>−2.5±2.2</td>
<td>7</td>
</tr>
<tr>
<td>F</td>
<td>O₄₋</td>
<td>41.1±3.0</td>
<td>−1.4±1.0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>O₃₋</td>
<td>79.3±3.4</td>
<td>−0.4±2.2</td>
<td>4</td>
</tr>
<tr>
<td>G</td>
<td>O₄₋</td>
<td>19.8±1.9</td>
<td>−9.5±2.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>O₃₋</td>
<td>27.3±6.6</td>
<td>−13.2±3.5</td>
<td>4</td>
</tr>
</tbody>
</table>

Bath solution was solution A (143 mM KCl). The reversal potentials were corrected for the liquid junction potentials. Solution D contained 145 mM NaCl, 5.4 mM KCl, and 0.5 mM CaCl₂; solution H, 122 mM K-aspartate and 18 mM KCl; solution E, 150 mM NaCl; solution F, 150 mM KCl; solution G, 110 mM CaCl₂.

In Fig. 6C, the extrapolated current through O₄₋ was inward at zero pipette potential and the extrapolated reversal potential in this particular patch was +7.1 mV. In eight patches under the same condition, the reversal potential of O₄₋ was +15.9±9.1 mV. In a different series of experiments, the membrane potential was measured using the nystatin-perforated patch clamp method. The membrane potential of the guinea-pig gastric muscle cells bathed in solution D varied among cells, averaging −12.9±7.1 mV (n=61). Thus, O₄₋ does not represent a leakage current passing through the seal.

The slope conductances and the reversal potentials for various pipette solutions are summarized in Table 2 (bath solution was always solution A). The I−V relationships for O₄₋ were obtained from the unitary current amplitudes at various patch potentials calculated by fitting the all-point histograms with Gaussian distributions. Both O₄₋ and O₃₋ seemed to be due to cation channels rather than anion channels because their reversal potentials did not depend on [Cl⁻]. As all the experiments in the present study were carried out in the cell-attached configuration, the exact composition of the internal solution was unknown. Nevertheless, a rough estimation of the permeability ratios of Na⁺, K⁺, and Ca²⁺ was performed using the constant-field theory described previously [19], assuming the intracellular permeable cation was 155 mM K⁺ alone (estimated from the reversal potentials with pipette solution F; 150 mM KCl). The permeability ratios of Pk:PNa:PCa were calculated to be 1:0.9:1.2 for O₄₋ and 1:0.9:1.0 for O₃₋.

Activation of SA channel by hypotonic solution

Application of hypotonic bath solution causes cell swelling and stretch of the cell membrane which could
activate SA channels. In the experiments using hypotonic solution (solution C), solution B was used as the control bath solution to keep the ionic composition constant. In the experiment shown in Fig. 7, application of the hypotonic bath solution induced channel activities, which seemed to be identical to the suction-induced O₅ and O₆. Out of 39 cells exposed to hypotonic solution, O₅ was induced in 28 cells and O₆ in 13 cells.

**DISCUSSION**

Guinea-pig gastric smooth muscle cells responded to suction with two distinct channel openings termed O₅ and O₆. O₅ had a short open-time and slope conductance of 33 pS under approximate physiological conditions with [Ca²⁺] of 0.5 mM. On the other hand, O₆ had a characteristic long open-time, a noisy open state and a conductance of 53 pS. Both O₅ and O₆ seemed to be due to cation channels. Estimated permeability ratios were $P_K:P_{Na}:P_{Ca}=1:0.9:1.2$ for O₅ and 1:0.9:1.0 for O₆. Taking errors of estimation into account, the channels exhibiting O₅ and O₆ did not seem to discriminate among K⁺, Na⁺, and Ca²⁺, thus they are both non-selective cation channels.

Distributions of open- and closed-times of O₅ could be fitted by the sums of two exponentials. As SA channels found in a variety of cell types have similar kinetics as O₅ in the present experiments, the channel representing O₅ seemed to be one of these ubiquitous SA channels [7, 20, 21]. On the other hand, the analysis of channel kinetics for O₆ was unsuccessful. As the channel activity induced either by the seal formation or by the continuous application of suction disappeared within several minutes, there seems to be some inactivation mechanism, which we did not examine in the present experiments. Although O₆ was not likely to represent leakage current passing through the seal, there remains the possibility that O₆ current might pass through the patch membrane via some non-channel pathway. However, stepwise openings and the gadolinium sensitivity of O₆ favor the idea that the current passes through channels rather than some other pathway.

The next question is whether O₅ and O₆ arise from two different kinds of SA channels or from two distinct open states of the single population of SA channels. We do not have strong evidence to answer this question, but it seems more likely that they are produced by a single population of SA channels, because in the present experiments, O₅ and O₆ coexisted in many of the patches examined and there were no patches exhibiting O₆ alone. As the threshold suction for O₆ was higher than that for O₅ even in the patches where we got only O₅, it is possible that O₆ may have been obtained at higher levels of suction than tested here.

Multiple openings responding to suction have also been reported in endothelial cells of porcine cerebral capillaries [22]. According to the authors, the three kinds of openings observed represent three different populations of channels. Although the high conductance SA channel in their study had a fairly long open-time resembling O₆ in the present experiment, it occurred rather rarely (in 15% of patches examined), it showed a relaxation in the current amplitude, and the open state was not noisy; all these characteristics were different from those of O₆.

An O₆-like long-lasting open state of the SA channel has also been observed in guinea-pig urinary bladder myocytes, when activated by bath-applied dibutyryl cAMP [21]. The authors concluded that the
phosphorylation of the channel protein by the cAMP-dependent protein kinase modulated the channel kinetics. In guinea-pig gastric smooth muscle cells, however, bath-applied dibutylryl cAMP did not significantly affect the SA channel activities (unpublished observation). Stronger suction, which induced O₂ in the present study, may affect the SA channel directly or indirectly via an intracellular mechanism other than that related to cAMP.

The SA channel could also be activated by introducing hypertonic bath solution in the cell-attached configuration. Hypotonic bath solution induced not only O₂ but also O₁ in the present experiment, indicating that stimulation by the 30% reduction in osmolarity was strong enough to induce O₁. In this condition, the patch membrane which incorporated the SA channels was not exposed to the bath solution. However, increased intracellular pressure induced by cell swelling might stretch the patch membrane and activate the SA channels directly. Alternatively, the SA channels might be activated indirectly via some mechanisms possibly related to cell swelling. SA channels may be activated by tension in the cytoskeleton, because patch membrane lipid is free to flow [23]. Cell swelling will modulate the tension in the whole cell cytoskeleton, thus activating the SA channels all over the cell membrane including those in the patch. Induction of the SA channel activity by cell swelling has also been observed in endothelial cells of porcine cerebral capillaries [22], in rat liver cells [20], and in frog renal epithelial cells [24]. In the cell-attached configuration, hypertonic stimulation also activated Cl⁻ channels in rabbit osteoclasts [25], in cultured human epithelial cells [26] and in cultured human skeletal muscles [27]. Although the long-lasting nature of O₂ in the present study resembled those of the Cl⁻ channels, O₁ was not due to Cl⁻ channels because its reversal potential did not depend on Cl⁻.

In conclusion, guinea-pig gastric smooth muscle cells have SA channels which are nearly equally permeable to Na⁺, K⁺, and Ca²⁺. With moderate membrane stretch, brief and repetitive openings (O₃) appear, introducing a small membrane depolarization and a small amount of Ca²⁺ influx. Stronger stretch induces longer openings (O₄) together with O₃, and introduces larger membrane depolarization and larger amounts of Ca²⁺ influx. Thus, these SA channels may play an important role in the stretch induced contraction.

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