Simultaneous monitoring of cellular depolarization and contraction: a new method to investigate excitability and contractility in isolated smooth muscle cells

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Sevcencu C, Pennisi CP, Yoshida K, Gregersen H. Simultaneous monitoring of cellular depolarization and contraction: a new method to investigate excitability and contractility in isolated smooth muscle cells. Am J Physiol Gastrointest Liver Physiol 294: G648–G654, 2008. First published January 10, 2008; doi:10.1152/ajpgi.00040.2007.—The present experiments were performed to establish a method for simultaneous monitoring of excitation and contraction in isolated smooth muscle cells. The smooth muscle cells were dissociated from the colon of Wistar rats and the force of contraction was measured simultaneously using intracellular microelectrodes and ultrasonic displacement transducers. The simultaneous recording of membrane depolarization and contraction was achieved by using ultrasonic displacement transducers as force transducers. The results demonstrate that this method can be used to investigate the electrical and mechanical properties of isolated smooth muscle cells.

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opposite end of the cell, which was used to record the stimulation artifact and to measure the amplitude and the force of cellular contraction. All the above-mentioned experiments were performed on mixtures of circular and longitudinal colonic cells.

MATERIALS AND METHODS

The smooth muscle cells were dissociated from the muscle layers of the descending colon of 16 2-mo-old male Wistar rats according to a protocol approved by the Danish Animal Welfare Committee.

Surgical and dissection procedures. The surgical, dissection, and dissociation procedures and the methods used to manipulate the isolated cells were described previously (10). Briefly, after the descending colon was removed and rinsed with saline, the muscular coat was dissected and cut into strips in a Petri dish filled with a 4°C Tyrode solution (in mM: 146 NaCl, 5 KCl, 1 MgCl2, 10 HEPES, 10 glucose). The muscle strips were then left to relax for 40 min at 37°C in Tyrode solution supplemented with bovine serum albumin (BSA, 4 mg/ml) and oxygenated with pure O2. The enzymatic digestion that followed lasted for 50 min and was carried out at 37°C in Tyrode solution oxygenated with pure O2 and supplemented with 4 mg/ml BSA, 2 mM CaCl2, 0.25 mM EDTA, 2 mg/ml papain, 1 mM 1,4-dithiothreitol, and 1 mg/ml collagenase type II. Dissociation was completed by gently stirring the partially digested strips and the cells were harvested by use of a fine nylon mesh. The cell suspension was kept at room temperature for the next 4–5 h and stored at 4°C overnight. Since both of the muscle layers of the colon were taken together, the cell suspension contained both longitudinal and circular muscle cells. However, since the circular layer is much thicker than the longitudinal layer, most of the cells used in the present study were probably circular muscle cells.

Drops of the cell suspension were placed in superfusion chambers (Falcon tissue culture dishes, Becton Dickinson Labware). The superfusion chambers were placed on the bridge of an immersion microscope (BX51WI, Olympus) and filled with Tyrode solution supplemented with 2 mM CaCl2. The cells were then left to disperse and attach to the bottom of the chamber for ~20–30 min. After this interval, the cells were washed by replacing the initial solution with fresh Tyrode solution 3–4 times.

Cell stimulation. Cell stimulation was achieved using bolus injections of 1 M KCl. The solution was injected in the bath toward the cell through a glass micropipette placed within 40–60 µm from the stimulated cells. The glass micropipettes used to deliver the bolus had external tip diameters of ~4 µm (Fig. 1B). The pressure to deliver the KCl bolus was 1 cm of water, and the bolus pulse was generated through a stopcock valve that was kept open for less than 1 s. To investigate which was the actual K+ concentration nearby the stimulated cells during 1 M KCl injection, potential changes induced in the Tyrode at several distances from the stimulation pipette were measured in control experiments. At distances of 10, 30, 60, and 80 µm from the tip of the stimulation pipette, the average potential changes in response to 1 M KCl injected as described above were 14.8, 11.6, 7.5, and 5.4 mV, respectively. In a different set of control experiments, we have measured potential changes induced by changing the K+ concentration in the Tyrode and concluded that the potential values measured at 10, 30, 60, and 80 µm from the tip of the stimulation pipette correspond to K+ concentrations of ~180, 110, 48, and 30 mM, respectively. These data indicate that the K+ concentration nearby the stimulated cells (40–60 µm from the tip of the stimulation pipette) was lower than 100 mM during 1 M KCl injection (see also the amplitude of the stimulation artifact in Fig. 4). In several other control experiments, the 1 M KCl solution in the stimulation...
pipette was replaced by Tyrode, which was delivered as described above.

**FEP recording.** To record the FEPs induced by chemical stimulation, one tip of the cell was aspirated into a pipette made of patch glass capillary (PG150T, 7.5, Harvard Apparatus). The pipettes were pulled with a P-97 Flaming/Brown micropipette puller (Sutter Instrument) and fire polished to a tip diameter of ~4 μm (tip resistance, 0.2 to 0.5 MΩ). The pipettes were filled with Tyrode solution and mounted onto a headstage (HS-2A, Axon Instruments) attached to a three-axis fluid-driven micromanipulator (Narishige). After the tip of the pipette was positioned near the tip of the target cell, a negative pressure of at least 1 cmH2O was used to aspirate the end of the cell into the pipette (Fig. 1A). The resistance of the seal between the pipette and the cell was assessed by measuring the passive membrane depolarization in response to 1-nA current pulses delivered through the recording pipette. FEP recording was performed in current-clamp mode via an AxoClamp 2B amplifier (Axon Instruments) and digitally sampled to a computer using a data-acquisition card (PCI-MIO-E4, National Instruments) and custom-written software sampling at 4 kHz. A total of 46 cells were used in this set of experiments.

**Simultaneous recording of FEP and AP.** FEPs and APs were simultaneously recorded from 16 cells. The FEPs were recorded as described above. The APs were recorded by using pipettes with a tip resistance of 30–40 MΩ, which were pulled from capillary glass (1.0 mm OD, 0.75 mm ID, A-M Systems). They were filled with 3 M KCl and mounted on a second HS-2A headstage. After one tip of the cell was aspirated in the FEP recording pipette, the AP recording pipette was inserted into the cell, close to the mouth of the FEP recording pipette (Fig. 1B). The pipette used for chemical stimulation and containing KCl was then advanced near the cell and stimulation was performed as described above. The positioning of the three pipettes relative to the cell was similar in all 16 experiments.

**Simultaneous recording of the stimulation artifact, FEP, and cellular contraction.** Cell stimulation and FEP recording were performed as described above. To record the stimulation onset and to monitor the cellular contraction, a flexible micropipette filled with 3 M KCl was inserted through the cell at the end opposite to that aspirated in the FEP recording pipette (Fig. 1C). This flexible micropipette had the same characteristics as the AP recording pipette and was connected to the second channel of the amplifier. The cell was then lifted from the bottom of the dish and gently stretched between the two pipettes. A total of seven cells have been used in this set of experiments.

Chemical stimulation induced cellular contraction, which was optically recorded with a digital camcorder (DCR-PC 330, Sony) attached through a camera port on the microscope. The digital video images were acquired to the computer and were later analyzed frame by frame to measure the contraction of the cell. The KCl bolus transiently changed the ionic concentration, the density, and optical index of the liquid surrounding the cell. This resulted in a simultaneous potential drop in the electrical circuit comprising the flexible micropipette and in an obvious halo visible around the cell. These two events were used to synchronize the electrical and video recordings and define time 0 in the two records.

The amplitude of cellular contraction was calculated as \( A = D - d \) (see Fig. 1 for illustration), where \( D \) is the distance between the tip of the flexible pipette and the tip of the FEP recording pipette prior to contraction, and \( d \) is the same distance in the consecutive video frames. To estimate the force of cellular contraction, the elasticity of the flexible pipettes was calibrated using an ultrasensitive force transducer (model 406A, Aurora Scientific). The force curves were obtained by plotting the calculated values against time.

The active mechanical work (W) performed by the cell during the contraction was calculated as \( W(t) = A(t) \times F(t) \), where \( A \) is amplitude, \( F \) is force, and \( t \) is time. The mechanical power curves were then calculated by taking the time derivative of the work curve. The peak contraction, force, work, and power were quantified and averaged. The average time curves for work and power were calculated by normalizing the work and power curves by the peak work and power performed by the cell before the curves were averaged across cells. This yields an average qualitative work and power profile performed by the average cell during its contraction. All values shown in this paper are means ± SE. The differences between the mean values were statistically analyzed by Student’s t-test.

**RESULTS**

The FEP: comparison with the AP. The FEPs and APs recorded from the rat colon cells consist of four phases: phase 0, the upstroke; phase 1, the partial repolarization; phase 2, the depolarization plateau; and phase 3, the final repolarization (Fig. 2). Some of the FEPs had a relatively large-amplitude depolarization plateau with a sharp (Fig. 2A) or smooth (Fig. 2B) transition from phase 2 to phase 3, and some others had a lower amplitude and dome-shaped depolarization plateau (Figs. 2B and 4B). Most of the FEPs recorded in the present study closely reproduced the shape of the APs recorded from the same cell (Fig. 2A). However, there were situations when the depolarization plateau of the AP was morphologically different compared with that of the simultaneously recorded FEP (Fig. 2B).

![Fig. 2. FEPs and APs simultaneously recorded from rat colonic cells. 0–3, phases of the signals.](http://ajpgi.physiology.org/Downloaded.html)
Table 1. Parameters of FEPs and APs recorded from smooth muscle cells dissociated from the colon of rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FEP (n = 46)</th>
<th>AP (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential, mV</td>
<td>2.8±0.5</td>
<td>-18.7±3.7</td>
</tr>
<tr>
<td>Spike amplitude, mV</td>
<td>44.5±2.3</td>
<td>65.7±5.1</td>
</tr>
<tr>
<td>Amplitude of the depolarization plateau, mV</td>
<td>8.9±0.7</td>
<td>29.1±4.4</td>
</tr>
<tr>
<td>Depolarization velocity, V/s</td>
<td>0.5±0.1</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Spike duration, s</td>
<td>1.5±0.2</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>Duration of the depolarization plateau, s</td>
<td>5.7±0.1</td>
<td>6.3±1.6</td>
</tr>
<tr>
<td>Total duration, s</td>
<td>13.9±2.1</td>
<td>13.2±2.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. FEP, focal extracellular potential; AP, action potential.

The values of the measured FEP and AP parameters are shown in Table 1. Significant differences between the two signals were observed with respect to the values of the resting potential, the spike amplitude, and the amplitude of the depolarization plateau (P < 0.05 for all comparisons). No significant difference between the two signals was observed with respect to the duration parameters.

Relation between FEP amplitudes and the seal resistance. The FEP spike amplitude increases nonlinearly with the value of the seal resistance (Fig. 3). Thus, for seal resistances larger than 2 MΩ, the amplitude of the FEP spike approaches values of the resting potential.

Cellular contraction and mechanical parameters. In the investigated cells, the maximum contraction amplitude was 8.7 ± 1.4 μm, which represents a cell shortening of 15% from an initial length of 58.7 ± 4.7 μm. The mean values of the investigated parameters are shown in Table 2. Two representative force curves are shown in Fig. 4, and the average normalized work and power curves are shown in Fig. 5.

Simultaneous recording of stimulation artifact, FEP, and cellular contraction. Figure 4 illustrates correlations between membrane depolarization and force development in two of the investigated cells. In both cases, membrane depolarization was almost instantaneous with the stimulation onset, and contraction of the cell started after a latency of ~60 ms. Although the FEP illustrated in the first example had a depolarization plateau with a relatively large amplitude (18 mV), the FEP in the second example had a dome shaped depolarization plateau of smaller amplitude (8.5 mV). The first depolarization pattern generates a biphasic contraction (C1 and C2 in Fig. 4A) and the second one a monophasic contraction with an interval of slower relaxation following a slight increase in the amplitude of depolarization plateau (the arrow in Fig. 4B).

When the investigated cells were flushed with Tyrode instead of KCl, no membrane depolarization occurred and the contractile response was absent.

DISCUSSION

This study shows that membrane depolarization in an isolated smooth muscle cell can be recorded with an extracellular pipette to which one end of the cell is attached by suction. If a second elastic pipette is inserted through the opposite end of the cell (Fig. 2), a biphasic contraction (C1 and C2 in Fig. 4A) and the second one a monophasic contraction with an interval of slower relaxation following a slight increase in the amplitude of depolarization plateau (the arrow in Fig. 4B).

Fig. 3. Relation between the amplitude of the FEP and the seal resistance. The scatterplot shows the measured values of the FEPs against the seal resistance. The interpolation line is a plot of Eq. 1, where the value of the FEP was obtained by varying the leak to patch resistance ratio from 0 to 4 and the term \( V_m - E_p \) (where \( V_m \) is the membrane potential and \( E_p \) is the net diffusional force for all ions across the membrane patch inside the recording pipette) was chosen to be 90 mV.

Fig. 4. Simultaneous recordings of KCl stimulation, FEP, and force development in 2 of the investigated cells (A and B). C1 and C2, phases of the contractile response; the double-head arrow indicates the maximum amplitude of the depolarization plateau; the small arrow indicates the start of a slight increase in the amplitude of the depolarization plateau.
the cell, this may be used to simultaneously monitor the stimulation onset and cell shortening and to estimate the force of cellular contraction.

**FEP as a descriptor of membrane depolarization.** The FEPs recorded from the rat colon cells are morphologically similar to gastric and intestinal APs recorded by others (1, 4, 14). As illustrated in Fig. 2 and Table 1, the FEP amplitudes represent a fraction of the AP amplitudes and depend on the seal between the cell and the recording pipette (Fig. 3). In our opinion, the similarities and differences between the FEPs and APs result from the common origin of these signals and can be illustrated by using a model of the FEP recording setup (Fig. 6A). In the simplified version of this model (Fig. 6B), the internal resistance of the cell \( R_i \) and the capacitors were neglected because \( R_i \) is far smaller than the resistance of the membrane outside the recording pipette \( R_m \) and the resistance of the membrane patch inside the recording pipette \( R_p \), and the electrical events in smooth muscle cells are much slower than the time constants of the membrane and pipette. In addition, control experiments with inactive cells attached to the recording pipette resulted in an insignificant potential increase. Therefore the contribution of this potential was also neglected. According to this model, KCl stimulation triggers a net membrane current \( I_m \), which passes entirely through the leak resistance \( I_m = I_l \) when the input current of the preamplifier \( I_m \) is clamped to zero \( I_m = 0 \). Hence, the recorded voltage measured by the recording pipette \( V_{\text{FEP}} \) can be expressed as a function of the membrane potential \( V_m \) by using the voltage divider equation

\[
V_{\text{FEP}} = (E_p - V_m) \frac{R_i}{R_i + R_p}
\]  

(1)

where \( R_i \) is the leak resistance. According to this equation, the net diffusional force for all ions across the membrane patch inside the recording pipette \( E_p \) is a constant offset for \( V_m \), which explains why the FEP is positive even when the cell is at its resting state (Table 1). In addition, Eq. 1 shows that \( V_{\text{FEP}} \) is a truncated version of \( V_m \) and the scaling factor depends on the ratio \( R_i/R_p \). Consequently, the larger the seal resistance, the higher the FEP amplitude, which is consistent with our experimental results (Fig. 3) and with the observations of Mason et al. (13), who showed that a seal-to-patch resistance ratio of 3 allows recording of 75% of a membrane potential across an intact cell membrane patch. It is also consistent with the model that most of the simultaneously recorded FEPs and APs have a similar shape (Fig. 2A). However, in some cases the shape of the AP differed from that of a simultaneously recorded FEP (Fig. 2B). This could be caused by mechanical instability of the cell-pipette interface during cellular contraction, which may have distorted the real AP morphology in some of the present recordings. If this assumption is confirmed by further experiments with isolated cells, then the FEP recording is a better option than using intracellular electrodes to assess qualitative aspects of membrane depolarization in contracting cells.

To increase the FEP amplitude and improve the FEP signal-to-noise ratio in frog cardiac cells, Riemer and Tung (15) used high K\(^+\) concentrations in the recording pipette and recorded FEPs with peak amplitudes of \( \sim 25 \) mV when using at least 70 mM K\(^+\). However, the cells that they used spontaneously fired APs when grasped with pipettes containing K\(^+\) at concentrations \( > 40 \) mM. To avoid any possibility of spontaneous depolarization and contraction of the cells in our experiments, we have investigated whether the FEP in these cells can be recorded by using normal extracellular K\(^+\) levels in the record-

![Figure 5](https://example.com/fig5.png)

Fig. 5. Average normalized instantaneous work (top) and power (bottom) curves generated by isolated colonic smooth muscle cells. The bold line indicates the average curve; the thinner lines indicate ±1 SD. The average peak work was 12.4 ± 4.9 pJ, and the average peak power was 36 ± 15 pW.

![Figure 6](https://example.com/fig6.png)

Fig. 6. Model of the FEP recording setup (A) and the simplified version of this model (B). \( C_m \), membrane capacitance; \( C_p \), capacitance of the membrane patch inside the recording pipette; \( C_{\text{pip}} \), pipette capacitance; \( E_m \), the net diffusional force for all ions across the membrane; \( E_p \), the electrochemical potential difference between pipette and bath; \( I_m \), the input current of the amplifier; \( I_l \), the leak current; \( I_{m0} \), the net membrane current; \( R_i \), the internal resistance of the cell; \( R_l \), the leak resistance; \( R_m \), the resistance of the membrane outside the recording pipette; \( R_{\text{pip}} \), the resistance of the membrane patch inside the recording pipette; \( V_{\text{FEP}} \), the voltage measured by the recording pipette.
ing pipette. As illustrated in Table 1 and Fig. 2, the FEPs recorded in this study have a relatively large amplitude and signal-to-noise ratio. This indicates that a high K⁺ concentration in the recording pipette is not a necessary condition to record FEPs with large signal-to-noise ratio in smooth muscle cells.

Independent of the shape of the recorded signals, the starts of the simultaneously recorded FEPs and APs were always instantaneous, the phases comprising the signals had almost identical durations, and no significant difference between the total duration of the FEPs and APs was observed (Table 1). This is consistent with the results of Riemer and Tung (15), who also found similar values for the duration of FEPs and APs recorded from frog cardiac cells.

In brief, our results show that the FEP accurately reflects the timing of membrane depolarization in smooth muscle cells, but the absolute values of the FEP amplitudes should be interpreted cautiously. However, for similar Rₓ values across the investigated cells, a condition that can be accomplished by manipulating the negative pressure in the recording pipette, the relative differences between the FEP amplitudes can be used to assess the effect of various factors on the magnitude of membrane depolarization.

Cellular contraction. The cells investigated in this study had an average length of ~60 μm and shortened with a maximum of 15% of the initial length. This is similar with the observations of Ferrier et al. (5) and Ma et al. (11), who worked with rat proximal and distal colon cells and reported that such cells shorten with 17 and 19% of initial cell lengths of 77 and 84 μm, respectively. In comparison, Sims (17) subjected canine gastric cells to acetylcholine (ACh) stimulation and observed a 33% maximum shortening of these cells 20 s after the application of the drug and a relaxation to 90% of the initial cell length 6 min later. In our experiments, the cells contracted almost 20 times faster (Table 2) and relaxed to 90.6% of the initial cell length 8 s after the peak contraction. Part of the differences between our results and those reported by Sims could be explained by physiological differences with respect to the species and the investigated cell types (rats vs. dogs, and colonic vs. gastric cells). In addition, it is likely that KCl stimulation triggers different activation mechanisms compared with ACh stimulation. Although it is known that ACh induces an elevation of the intracellular calcium concentration [Ca²⁺], through second messengers activation (6, 16), KCl stimulation may activate faster [Ca²⁺], elevation.

The colonic cells investigated in the present experiments developed an average force of 1.2 μN at peak contraction, which resulted in an average mechanical work of 12.4 pJ and an average peak power of 36 pW. Since work is a function of position and force, and force is a dependent function of position, the work curve resembles the average force and average contraction curve (Fig. 5). The peak power is derived at the steepest part of the work curve and is susceptible to jitter in the point where each individual cell reached its maximum power. This is reflected in the average curve not reaching 100% (Fig. 5). Our force measurements are consistent with the results of Harris and Warshaw (8), who reported that toad gastric cells develop a maximum force of 1.5 μN, and with the results of Brozovich and Yamakawa (2), who measured forces of 1.2–1.8 μN in isolated hog arterial cells. With respect to the values calculated for the velocity of contraction and relaxation, mechanical work, and power production, we could not find data regarding similar measurements in isolated smooth muscle cells. Hence the present monitoring method is capable of recording contractile activity of isolated smooth muscle cells and provides novel information regarding the dynamics and magnitude of cellular contraction for rat colonic cells.

Aspects of the ECC illustrated by simultaneous monitoring of cell stimulation, depolarization, and contraction. In the present recordings, the start of membrane depolarization was almost instantaneous with the onset of KCl stimulation (Fig. 4). This indicates that KCl stimulation almost instantly triggers inward depolarizing currents, most probably calcium currents through L-type channels (17, 18, 20). Consistently, the short latency (up to 60 ms) and fast contraction (8.2 μm/s) observed in this study suggest fast [Ca²⁺], elevation and cross-bridge formation in the investigated cells. This is similar with the results of Ozaki et al. (14), who observed a maximum level of contraction in canine gastric strips 0.3 s after a [Ca²⁺], elevation that starts almost instantaneous with the onset of membrane depolarization.

In this study, we have recorded both biphasic and monophasic contractions (Fig. 4). As mentioned before, the present experiments were conducted with a mixture of circular and longitudinal muscle cells and it is likely that these two patterns of contraction belong to the different cell types that we used. Indeed, biphasic contractions were recorded by Martin et al. (12) from circular strips dissected from the colon of rats, and monophasic contractions were recorded by Grider (7) from colon longitudinal strips of the same species. With respect to these two types of contraction, our recordings show that the contractile activity in the investigated cells is correlated with the characteristics of the membrane depolarization. Thus the FEP triggering and supporting the biphasic contraction has a large-amplitude depolarization plateau (Fig. 4A), whereas the monophasic contraction is triggered and supported by an FEP with a low-amplitude depolarization plateau (Fig. 4B). In the latter case, the inhibition of relaxation followed and was probably due to a slight increase in the amplitude of the FEP depolarization plateau (Fig. 4B). These observations are consistent with the results of Ozaki et al. (14), who showed that the APs recorded from myenteric muscles of the canine stomach have a large-amplitude depolarization plateau and support biphasic contractions. In contrast to this, submucosal gastric muscles generate APs with low-amplitude depolarization plateaus that support monophasic contractions (14).

In conclusion, the FEP recording can be used to investigate membrane depolarization in smooth muscle cells, and the method developed to monitor contraction of these cells accurately illustrates their mechanical activity. Combination of these two methods resulted in a new technique to simultaneously monitor excitability and contractility in smooth muscle cells as a method to investigate correlations between excitation and contraction in such cells.

REFERENCES


