Determination of Ca\textsuperscript{2+}–release from the Isolated Smooth Muscle Cells in Suspension from the Guinea-pig Ileum

Tsutomu URUNO, Atsushi WATANABE, Koh-ichiro TAKAHASHI, Nobuyoshi SUNAGANE, Yutaka MATSUOKA and Kazuhiko KUBOTA

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Science University of Tokyo, Ichigaya Shinjuku-ku, Tokyo 162, Japan

Abstract


Collagenase-dispersed cells from the guinea-pig ileum were prepared and Ca\textsuperscript{2+} release into Ca\textsuperscript{2+}–depleted solution from the isolated single cells obtained by the centrifugation of the dispersed cells on isotonic sucrose solution was determined with a Ca\textsuperscript{2+}–selective electrode. A technique employing an isotonic sucrose solution for washing isolated cells permitted removal of contaminating extracellular fluids and obtaining the isolated cells with minimum loss of cellular Ca\textsuperscript{2+}. The release of Ca\textsuperscript{2+} from the dispersed cells consisted of at least two phases. The Ca\textsuperscript{2+} release into an isotonic sucrose-Tris solution (ISTS) was significantly reduced and the early phase of the Ca\textsuperscript{2+} release disappeared. At 16°C, Ca\textsuperscript{2+} release depended on the composition of the bathing solution in a Ca\textsuperscript{2+}–free salt solution, the later phase of Ca\textsuperscript{2+}–release was abolished whereas in ISTS both phases disappeared. Furthermore, Ca\textsuperscript{2+} release was significantly reduced after the treatment of the dispersed cells with dispase (1,500 units/ml) for 10 min. These results show that Ca\textsuperscript{2+} release into Ca\textsuperscript{2+}–depleted solution from the isolated ileal cells with minimum loss of cellular Ca\textsuperscript{2+} show a biphasic curve and suggest that Ca\textsuperscript{2+} sources responsible for these phases may be of distinct origin.

Introduction

It is difficult to measure Ca\textsuperscript{2+} fluxes accurately in the smooth muscle tissue because of the complex extracellular compartment and of large extracellular space of smooth muscle. To obviate this difficulty, some attempts have been made to remove the extracellular Ca\textsuperscript{2+}, including a reduction of Ca\textsuperscript{2+} concentration in a medium and addition of agents such as La\textsuperscript{3+} and EGTA to a Ca\textsuperscript{2+}–free medium. Recently, isolated and dispersed smooth muscle cell suspensions have been prepared for measurement of Ca\textsuperscript{2+} fluxes (Hirata et al., 1981; Momose et al., 1983; Scheid and Fay, 1984a, b). On the other hand, since it has been suggested that the mobilization of bound Ca of an extracellular pool which supplies Ca\textsuperscript{2+} during the early states...
of activation be of importance (Lodge and Van Breemen 1985), it is significant to study Ca\textsuperscript{2+} fluxes from the isolated single smooth muscle cells with little loss of cellular calcium, including surface bound calcium, but these procedures are extremely difficult. Sanui and Rubin (1979) reported that repeated rinses of the chicken embryo fibroblast cells grown in culture with CO\textsubscript{2}-free 0.25 M sucrose solution produce essentially no loss of cellular cations. It was expected that this method would also be applicable to the isolated smooth muscle cells.

In the present study, we prepared collagenase-dispersed cells from the guinea-pig ileum, and investigated Ca\textsuperscript{2+} release into Ca\textsuperscript{2+}-deficient solution from the isolated single cells obtained by the technique employing a sucrose washing medium, i.e. the centrifugation of a cell suspension on isotonic sucrose solution in order to remove the medium contaminants. The Ca\textsuperscript{2+} ions released from the cells were determined with a Ca\textsuperscript{2+}-selective electrode. Our results suggest that the Ca\textsuperscript{2+} release from the isolated cells from the guinea-pig ileal longitudinal smooth muscle layers consist of at least two phases, an early phase dependent upon extracellular Na\textsuperscript{+} and a later one dependent upon temperature.

**Materials and methods**

*Ileal longitudinal smooth muscle preparation and experimental media*

Male guinea-pigs weighing 250–400 g were used in all experiments. Media used in the present experiments and their abbreviations are listed in Table 1. All the media except isotonic sucrose solution (ISS) were adjusted to pH 7.4 with HCl and saturated with 100% oxygen.

Animals were stunned by a blow on the head and bled. The abdominal cavity was exposed and the entire small intestine was removed. The terminal ileum (about 10 cm immediately before the caecum) was discarded. An approximately 6 cm long section was inserted with a glass rod (0.6 cm in diameter) and a gentle incision was made along the line where mesentery was attached. With the help of a piece of cotton wool soaked in physiological solution (NTS or BTS), the longitudinal muscle layer was separated from the underlying circular muscle by gently stroking it away from its mesenteric attachment along the entire segment.

**Measurement of contractile responses**

It is desirable to minimize the effects of ionic strength of the solution on the cell activity

<table>
<thead>
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<th>Media</th>
<th>Na\textsubscript{Cl}</th>
<th>K\textsubscript{Cl}</th>
<th>Ca\textsubscript{Cl\textsubscript{2}}</th>
<th>Glucose</th>
<th>Tris</th>
<th>Sucrose</th>
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<tr>
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<td>1.8</td>
<td>5.6</td>
<td>23.8</td>
<td>113.8</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}-free BTS</td>
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<td>2.7</td>
<td>0</td>
<td>5.6</td>
<td>23.8</td>
<td>113.8</td>
</tr>
<tr>
<td>Low Ca\textsuperscript{2+} BTS</td>
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<td>2.7</td>
<td>0.18</td>
<td>5.6</td>
<td>23.8</td>
<td>113.8</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>23.8</td>
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</tr>
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</table>

Note: Abbreviation used: NTS, normal 125-mM Na\textsuperscript{+}, Tris-buffered solution; BTS, basic 60-mM Na\textsuperscript{+}, Tris-buffered solution; ISS, isotonic sucrose solution; ISTS, isotonic sucrose–Tris solution.
when enzymatically isolated smooth muscle cells are washed in ISS or ISTS to remove extracellular ions. Therefore, we decided to reduce the Na⁺ content in the physiological salt solution (NTS) to about a half, and contractile responses to carbachol in 60 mM Na⁺ salt solution (BTS) were compared with those in 125 mM Na⁺ solution (NTS). Ileal longitudinal layer segments (1.5-2 cm long) were placed in thermostatically-controlled 10 ml glass tissue baths containing NTS or BTS at 32°C and gassed continuously with 100% O₂. Tension of 0.5 g was applied to the segments and isotonic contractions were detected by an isotonic transducer (ME-4012, ME Commercial Co. Ltd., Tokyo) and recorded on a paper recorder.

The muscle was suspended in the organ bath for 30 min before the addition of carbachol. During this period, it was washed several times with NTS. A cumulative contractile dose-response curve for carbachol was obtained by a stepwise increase in concentration after a steady response occurred to the preceding dose. On completion of the curve the tissue was washed 5 times at 3-min intervals. After reproducible curves were obtained in NTS, the bathing medium was switched to one containing 60 mM Na⁺ (BTS), the tissue was incubated for 15 min in the BTS, and three concentration-response curves in BTS were then obtained for carbachol at 15-min intervals.

Cell preparation

Enzymatical digestion of isolated longitudinal smooth muscle layers of the guinea-pig was carried out by a modification of Momose and Gomi (1978). A segment of the longitudinal smooth muscle layer (2-3 cm, about 1.5 g wet wt) was placed in BTS bubbled with air at 37°C for 30 min, transferred into low Ca²⁺-BTS, and incubated for further 30 min. The segment was then cut into small pieces and digested with stirring 160 times/min for 60 min in 10 ml low Ca²⁺-BTS containing 0.2% collagenase, 0.2% trypsin inhibitor and 1.0% bovine serum albumin. After the digestion, the undigested tissue was transferred to a glass centrifugal tube, allowed to stand for 3 min and the supernatant was discarded. The residual tissue was resuspended in 10 ml of low Ca²⁺-BTS saturated with 100% O₂. Thereafter all the solutions used were saturated with pure O₂. The muscle pieces were twice washed with the low Ca²⁺-BTS, resuspended in 10 ml of the solution, and incubated in an incubator (100 oscillations/min) at 37°C for 60 min. At the end of the incubation, 10 ml of the low Ca²⁺-BTS was added and the remaining muscle pieces were subjected to mild pipetting by slowly passing them through a wide-bore (3 mm) siliconized pipette and filtering the pieces over nylon mesh. The 20 ml filtrate containing the suspension of isolated smooth muscle cells was layered on top of the freshly prepared discontinuous Ficoll density gradients (7 and 15% Ficoll in BTS, 10 ml each) and centrifuged at room temperature (22-26°C) at 30×g for 5 min, subsequently at 100×g for 10 min. The smooth muscle cell layer at the interface was gently removed with a pipette and resuspended in 72 ml low Ca²⁺-STS. This suspended solution was stored in 8-ml aliquots at room temperature for future use.

Cell viability was assessed by both trypan blue dye exclusion and retention of lactic dehydrogenase (LDH). For determination of the trypan blue exclusion, 2 ml of dispersed cells (in BTS) was layered on 5 ml isotonic sucrose solution (ISS) in a 10 ml centrifuge glass tube and centrifuged at 50×g for 5 min at room temperature. The supernatant was then removed, 50
μl BTS was added and the cells were resuspended. The suspension was combined with 100 μl of a 0.4% trypan blue solution dissolved in BTS for 5 min and the isolated cells which were able to exclude trypan blue were counted in a hemocytometer.

For determination of LDH activity, dispersed cells prepared after the Ficoll density gradient centrifugation were incubated for 30 min or 90 min at 37°C in a shaking water bath. After centrifugation at 50×g for 5 min at 26°C, the cell pellet was suspended in 5 volumes of BTS and homogenized with a Polytron homogenizer PT 10 (Kinematica, Luzern, Switzerland) for ten 5-sec bursts with cooling (4°C) in between at a maximum speed. Ten volumes of BTS were then added to the homogenate and stored at 4°C. Aliquots were used for determination of LDH activity in the supernatant after centrifugation at 100×g for 10 min, and also used for protein determination. The LDH determination based on the method of Wroblewski and LaDue (1955) was performed using a Sigma kit (Sigma Chemical Co., St. Louis, Missouri). The procedure for the LDH determination is described in Sigma Technical Bulletin No. 340-UV.

Protein was measured by the modified Lowry method (Schacterle and Pollack, 1973), using bovine serum albumin as a standard.

**Ca²⁺ release from tissue and dispersed cells**

The determination of Ca²⁺ release was carried out as described previously (Uruno et al., 1981; Shiba et al., 1981). The potentiometric set-up for Ca²⁺ is composed of an F2112Ca Calcium Selectrode, a K401 saturated calomel electrode, a PHM 64 pH meter (Radiometer A/S, Copenhagen, Denmark) and a recorder with an adjustable zero offset. The calibration curve for Ca²⁺ was made before and after the release experiments to minimize possible errors due to the electrode potential drift. Free Ca²⁺ measurements were carried out in a Radiometer TTA 60 Titration Assembly.

After an isolated longitudinal smooth muscle layer was allowed to equilibrate at 32°C for more than 60 min in BST, the layer was gently blotted on ashless filter paper (Whatman No. 42), rinsed in 20 ml ISS or ISTS for a given time, and then placed in a vessel containing Ca²⁺-free BST (2 ml) to continuously determine Ca²⁺-release from the tissue during a given period by the Ca²⁺-electrode system. Stirring of an efflux medium was accomplished by bubbling of 100% O₂. After Ca²⁺ determination, the tissue was blotted and dried in an oven at 110°C for 12 hr to obtain the dry wt. Ca²⁺ ions released were expressed as nmols/kg dry wt.

For determination of Ca²⁺ release from dispersed cells into Ca²⁺-free BTS, 2 ml of cell suspension was layered on isotonic sucrose solution (ISS) and centrifuged at 50×g for 10 min to separate the cells from the solution containing Ca²⁺. The supernatant was discarded and the cell pellet was resuspended in 200 μl ISS. Ca²⁺ release from the cells were continuously recorded by adding the resuspension of 200 μl to 2 ml Ca²⁺-free BTS, which had been saturated with pure oxygen at 26°C. The determination medium was stirred at 100 rpm with a stirrer (No. 847-713, TTA 60, Radiometer). After determination of Ca²⁺ release, protein content in the medium was determined and Ca²⁺ concentration released was expressed nmols/mg protein.
Statistics
Statistical evaluations were made by Student’s t test. Values are mean±SE.

Materials
Collagenase Type 1 (Sigma Chemical Co., St. Louis, Missouri), soy bean trypsin inhibitor (Sigma Chemical Co., St. Louis, Missouri), carbamylcholine chloride (Tokyo Kasei Kogyo Co. Ltd., Tokyo), iodoacetic acid (sodium salt) (Sigma Chemical Co., St. Louis, Missouri), dispase II (Godo-Shusei Co. Ltd., Tokyo), and Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden). All the other chemicals were of reagent grade, and all solutions were made using reagent–grade water processed through Milli-Q systems (Millipore Corp., Bedford). All glassware used was siliconized. Experiments, unless otherwise stated, were carried out at room temperature (22–26°C).

Results
Effect of 60 mM Na+ medium on contractile response
In the present experiments, we used 60–mM Na+, Tris-buffered solution (BTS) as basic physiological solution in order to minimize the effect of changes in ionic strength by the centrifugation of an isolated smooth muscle cell suspension on isotonic sucrose solution (ISS). The responses of the guinea-pig ileal longitudinal smooth muscle to carbachol in the medium containing 120 mM Na+ (NTS) were compared with those in BTS. Fig. 1 shows the responses to carbachol after NTS was changed to BTS. The concentration–response curves for carbachol were shifted to the right and the maximal responses were inhibited by changing NTS to BTS, but the reproducible responses to carbachol were obtained after incubation for more than 45 min in BTS. These results suggest that in BTS reproducible dose–response curves similar in shape to those in NTS although sensitivity and responsiveness of the tissue were reduced.

Fig. 1. Effect of 60 mM Na+ medium (BTS) on contractile responses of ileal longitudinal smooth muscle layers to carbachol. After a control concentration–response curve (∙) was obtained in 125 mM Na+ solution (NTS), the bathing solution was switched to BTS and three concentration–response curves, first (∙), second (▲) and third (∨) challenges, were constructed. The medium was then returned to NTS and the fourth curve (∗) was obtained in NTS. Each curve represents the average of six experiments, and vertical bars indicate SE. Details in Materials and methods.
Ca\(^{2+}\) release from smooth muscle layers

The Ca\(^{2+}\) release from the longitudinal smooth muscle layer of the guinea-pig into Ca\(^{2+}\)-free solution (Ca\(^{2+}\)-free BTS) was determined after the tissue was rinsed for a given time in ISS or ISTS. Fig. 2 shows the Ca\(^{2+}\) release from ileal longitudinal smooth muscle layers into Ca\(^{2+}\)-free BTS. There were no significant differences between the concentrations of Ca\(^{2+}\) ions released from the tissues rinsed for 30 sec, 3 min and 10 min in ISS (Fig. 2A), while a time-dependent decrease in the Ca\(^{2+}\) release was observed after rinsing the tissue in ISTS (Fig. 2B). Rinsing the tissue in Ca\(^{2+}\)-free BTS produced a much greater loss of Ca\(^{2+}\) release compared with the release in ISTS (data not shown).

Viability of isolated smooth muscle cells

The isolated cells obtained are generally spindleshaped in form but vary somewhat in length (data not shown). Microscopic evidence showed that our preparation consisted almost entirely of single cells. The viability of the isolated smooth muscle cells prepared in the present study was examined in terms of the trypan blue exclusion and retention of lactic dehydrogenase (LDH). For the determination of the trypan blue exclusion, over 87% of cells (87.6±0.9, n=17) had the ability to exclude the dye. After completion of the experimental procedures, more than 86% of the cells (86.1±0.9, n=17) still retained resistance to the dye. Intracellular LDH activities were measured after the incubation of cell suspension for 30 and 90 min. The respective values were 3,496±97 (n=3) and 3,593±182 units/mg protein (n=3), and no significant difference was observed between them. This shows that the leakage of LDH from the cells was negligible. From these results, we have decided the isolated smooth muscle cells used in the present study are applicable to the analysis of properties of Ca\(^{2+}\) release.
from these cells.

**Ca**<sup>2+</sup> release from dispersed smooth muscle cells and effects of agents and temperature

Fig. 3 shows a typical response of a Ca**2+**-selective electrode to Ca**2+** concentration released from isolated cells. The concentration of cells used was 0.8×10⁶ (=0.53 mg protein/ml). The release of Ca**2+** from the dispersed cells consisted of at least two phases. It may be seen that initially the Ca**2+** was released rapidly and it reached a peak in 1–2 min, and that after that time the Ca**2+** released reached a near-maximum in the subsequent 8–10 min (Fig. 3).

The release of Ca**2+** from the isolated cells treated with iodoacetic acid (IAA) into Ca**2+**-free BTS is shown in Fig. 4. After the treatment with IAA (10⁻³ M) for 10 min, the cells were washed by a centrifugation on isotonic sucrose solution. IAA was added to the cell suspension in the presence of Ca**2+**. The concentration of Ca**2+** released during 10-min incubation was doubled by the IAA treatment as compared with that of the control. The typical biphasic release of Ca**2+** disappeared after the IAA treatment.

The release of Ca**2+** from the isolated cells and tissue after their pretreatment with isotonic 40 mM K⁺ for 5 min was illustrated in Fig. 5. The 40 mM medium was made by subtracting 40 mM NaCl from BTS and adding 40 mM KCl. The Ca**2+** release from the tissue was measured after rinsing it in ISTS for 3 min. The release of Ca**2+** from both the tissue (Fig. 5A) and the isolated cells (Fig. 5B) was significantly increased by pretreatment with high K⁺. However, the significant increase in the Ca**2+** release from the tissue was observed only in the later phase (Fig. 5A). No significant increase in the Ca**2+** release from both the ileal smooth muscle layer and
isolated cells treated with carbachol (10^{-5} M) for 5 min was observed (data not shown).

Fig. 6 shows the release of Ca^{2+} from the isolated cells into ISTS at room temperature (22-26°C), Ca^{2+}-free BTS at 16°C, and ISTS at 16°C. The Ca^{2+} release into the ISTS was significantly reduced and the early phase of the Ca^{2+} release disappeared (Fig. 6A). The release

![Graphs showing release of Ca^{2+}](image)

Fig. 5. Effect of pretreatment with 40 mM K+ on release of Ca^{2+} ions from ileal longitudinal smooth muscle layers (A) and isolated and dispersed smooth muscle cells (B). After tissues and isolated smooth muscle cells were pretreated 40 mM K+ for 5 min, the tissues were rinsed for 3 min in ISTS and the isolated cells were washed by the centrifugation on ISS. (○): control, (●): 40 mM K+. The symbols represent mean±SE. (n=6 for A and n=3 for B). * and **, significantly different from control values at P<0.05 and P<0.01, respectively. The abbreviations are shown in Table 1.

![Graphs showing release of Ca^{2+}](image)

Fig. 6. Release of Ca^{2+} from isolated cells into ISTS at room temperature (22-26°C) (A), Ca^{2+}-free BTS at 16°C (B), or ISTS at 16°C (C). (○): control (Ca^{2+}-free BTS at room temperature), (●): ISTS (A), Ca^{2+}-free BTS at 16°C (B), or ISTS at 16°C (C). Each curve represents the average of 3 determinations, and vertical bars indicate SE. The abbreviations are shown in Table 1.
Ca^{2+}-release from isolated smooth muscle cells in suspension

Fig. 7. Effects of NTS (A) and dispase (B) on release of Ca^{2+} from isolated cells in suspension. (○): control, (■): 125 mM Na^+ solution (NTS)-treatment for 5 min or dispase (1,500 units/ml)-treatment prior to Ca^{2+} release measurements. The vertical bars represent mean SE (n=3).

Discussion

The collagenase-dispersed and Ficoll-purified smooth muscle cells from the guinea-pig ileum used in the present experiments appeared to consist of single cells and be viable under microscopic observation. We used two independent assays of plasma membrane integrity to obtain the actual confirmation of viability of the cells. One was a trypan blue exclusion test and more than 87% of the isolated cells excluded trypan blue. The other was a determination of intracellular LDH activity and the isolated cells showed absence of leakage of intracellular LDH. The results of these assays suggest that the cells may viable and retain a functional plasma membrane.

It is necessary to remove Ca^{2+} from a medium containing dispersed cells in order to determine the Ca^{2+} release from the dispersed cells into a Ca^{2+}-free medium. Washing the cells in a Ca^{2+}-free physiological salt solution has been shown to cause rapid loss of cellular calcium bound loosely and tightly on the cell membrane. Sanui and Rubin (1979) have reported that rinse of the cultured cells of chicken embryo fibroblasts with 0.25 M sucrose solution enables to remove the medium contaminants essentially with little loss of cellular cations. Therefore, we tried to use isotonic sucrose solution (ISS) as a washing solution and we have
confirmed that rinsing of the ileal longitudinal smooth muscle layer in ISS and washing of the dispersed smooth muscle cells by the centrifugation on ISS are more satisfactory to minimize loss of cellular calcium compared with electrolytic medium treatment. We determined Ca\(^{2+}\) concentration released from the single cells in suspension into a Ca\(^{2+}\)-free medium using a Ca\(^{2+}\)-selective electrode. The feasibility of using a Ca\(^{2+}\)-selective electrode for measurements of Ca\(^{2+}\) movements with mitochondria (Chance and Yoshida, 1966; Crompton et al., 1978; Yamazaki et al., 1979), sarcoplasmic reticulum (Madeira 1975), and smooth muscle preparations (Urano et al., 1981) has been demonstrated.

Treatment of isolated smooth muscle cells from the guinea-pig ileum with iodoacetic acid (IAA) in BTS or isotonic high K\(^+\) (40 mM) greatly increased Ca\(^{2+}\) release into a Ca\(^{2+}\)-free medium. The increased Ca\(^{2+}\) release may be caused by an elevation of the internal Ca content of the cells. It has been reported that the tissues exposed to IAA and dinitrophenol develop membrane leakiness and maintain high level of Ca (Casteel et al., 1972; Brading, 1978), and that the single cells in suspension from the guinea-pig taenia coli increase \(^{45}\)Ca uptake with 3–5 min exposure to K-rich solution (Hirata et al., 1981). The dispersed single cells used in the present experiment were responsive to the stimuli, suggesting that they are viable and retain a functional plasma membrane which is selectively permeable to Ca\(^{2+}\) ions.

We then investigated the effects of several treatments on Ca\(^{2+}\) release curve from the single cells in suspension from the guinea-pig ileum. The Ca\(^{2+}\) released from isolated single cells prepared by a technique employing a sucrose washing media into Ca\(^{2+}\)-free solution showed a curve with two phases: the initial one reached a peak in 1–2 min and the subsequent one did a near-plateau in 8–10 min. The failure of the whole tissue to show the biphasic response in its Ca\(^{2+}\)-release is not clear, but a possible explanation may be that the complex extracellular component, large fraction of extracellular Ca\(^{2+}\) and the diffusional barrier in the whole tissue make the Ca\(^{2+}\)-release from the cells obscure. The Ca\(^{2+}\) release into isotonic sucrose-Tris solution (ISTS) was inhibited and the initial phase disappeared, suggesting that it may be dependent upon Na\(^+\) in a Ca\(^{2+}\)-determining medium. Meanwhile, the Ca\(^{2+}\) release into Ca\(^{2+}\)-free solution (Ca\(^{2+}\)-free BTS) at 16°C produced inhibition of the later phase. In addition, both phases disappeared when the Ca\(^{2+}\) release into ISTS at the lowered temperature was determined. The first phase of the Ca\(^{2+}\)-release curve was also intervened by the treatment of the single cells in suspension with 125 mM Na\(^+\) solution (NTS) or dispase (1,500 units/ml). Thus, the Ca\(^{2+}\) release curve with the two phases was differently influenced by the various treatment procedures of the isolated smooth muscle cells, suggesting that the Ca\(^{2+}\) sources responsible for the initial and later phases may be of distinct origin, i.e. the initial phase of the Ca\(^{2+}\) release from the cells in suspension may be extracellular Na\(^+\)-dependent and the second one temperature-dependent. These results also indicate that the Ca\(^{2+}\) released in the early phase may originate from the surface Ca\(^{2+}\) and/or Na\(^+\) binding sites of the cells which are sensitive to dispase.

Dispase is a neutral and zinc-containing protease (EC 3.4.24.4) derived from Basilllus polymyxa (Morihara et al., 1968). Dispase II describes a partially purified preparation. The protease has been used for dispersion of tissues, for transferring monolayer cultures and maintaining cells in suspension, and favorable papers on dispase in isolating pancreatic cells.
Ca²⁺-release from isolated smooth muscle cells in suspension

with high viability as a single cell suspension and its non-toxicity on cells have been presented (Ono et al., 1977; Thilo et al., 1980). However, it is unclear whether the protease modifies the Ca²⁺ and/or Na⁺ binding sites or other regulatory membrane constituents, resulting in its inhibitory effects on the smooth muscle cell activity. Our preliminary studies showed that the contractile responses of isolated ileal longitudinal smooth muscle of the guinea pig to 40 mM K⁺ and 10⁻⁶ M carbachol were inhibited by treatment with dispase (50 units/ml) by about 50% (data not shown). Therefore, special consideration should be given to the effect of dispase on the smooth muscle when the tissue is digested enzymatically in the presence of dispase.

In summary, the technique employing the centrifugation of ileal smooth muscle cell suspension on isotonic sucrose solution prior to measurement of Ca²⁺ release may permit to remove contaminating extracellular fluids and to obtain the isolated cells with minimum loss of cellular Ca²⁺. Our data suggest that Ca²⁺ release from the isolated single cells prepared by the technique employed here show a biphasic curve; the initial phase may be extracellular Na⁺-dependent and dispase-sensitive, and the later phase temperature-dependent.

References


