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Studies on Isolated Smooth Muscle Cells. III.[1] Calcium Contraction of Isolated Cells from *Vas Deferens* of Guinea Pig

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Mode of calcium contraction of isolated smooth muscle cells from *vas deferens* of guinea pig was investigated. The cells were prepared by treatment of the *vas deferens* with collagenase followed by mechanical agitation. The isolated cells were contracted by administration of calcium ions under the depolarized condition. Degree of the contraction in each cell was not dependent on concentration of calcium but it seemed to be all-or-none like response while contraction of the whole tissue was graded response. The each cell had different sensitivity to calcium ions and total number of contracted cells increased with increase in concentration of calcium in the incubation medium. Rate of the contraction in each cell was dependent on temperature. The contraction was inhibited by ruthenium red but not by 2,4-dinitrophenol. The cells were also contracted by strontium and barium ions.

Keywords——smooth muscle cells; *vas deferens*; calcium contraction; isolated cells; all-or-none like response

In the previous paper,[1] the present authors reported that isolated smooth muscle cells from *vas deferens* of guinea pig could be contracted by calcium ions and the calcium contraction of partially depolarized cells were potentiated by cocaine. It was also observed that, when a given concentration of calcium were administered to the isolated cells, each cell contracted either completely or slightly and halfly contracted cells were rare. In order to confirm the facts, further investigation was carried out. In this paper the authors present some characteristics of the calcium contraction with isolated smooth muscle cells of guinea pig *vas deferens*.

Experimental

Materials——Collagenase was obtained from Sigma Chemicals (Type I) and ruthenium red was from Schmid and Co. (untertürkbeim). Other chemicals were guaranteed grade.

Isolation of Single Smooth Muscle Cells from *Vas Deferens* of Guinea Pig——Single smooth muscle cells were isolated from *vas deferens* of male albino guinea pig (350—400 g) by treatment with the collagenase in the presence of 1.0% bovine plasma albumin as previously reported.[3]

Contraction of the Single Smooth Muscle Cells——The isolated cells were perfused continuously with incubation medium (140 mM KCl, 1.0 mM $MgCl_2$, 5.6 mM glucose and 10 mM Tris HCl, pH 7.4) on dichlorodimethylsilane-coated slide glass at 30 or 37° and contraction was observed with use of phase contrast microscope as previously reported.[3]

Results

Calcium Contraction of *Vas Deferens*

Prior to examination with the isolated smooth muscle cells of *vas deferens*, calcium contraction of the muscle as a whole tissue was examined under the same condition as applied

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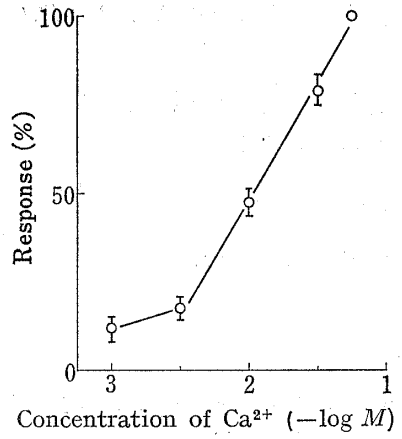

Fig. 1. Dose-Response Relationship for Calcium Contraction of *Vas Deferens* as Whole Tissue

Vas deferens of guinea pig was suspended in calcium free modified Tyrode solution (2.7 mM KCl, 137 mM NaCl, 1.0 mM $MgCl_2$, 5.6 mM glucose and 6.0 mM $NaHCO_3$) and tension was loaded. It was allowed to stand for 60 min at 35°, then the medium was replaced by the incubation medium. The tissue was contracted, first, by 7.8×10^{-3} M $CaCl_2$ twice, then it was contracted by cumulative administration of calcium chloride to obtain dose-response relationships. The contraction was recorded isotonically. Vertical bar represents standard error of mean ($n=6$).

to the isolated cells. This calcium contraction occurred promptly by addition of calcium chloride to the incubation medium and completed soon. Fig. 1 shows the dose-response relationship for the calcium contraction. Concentration more than 3.2×10^{-3} M was effective to induce the significant contraction and 5.6×10^{-1} M was maximum concentration to induce the rapid contraction.

Response of Each Cell to Calcium

In order to examine sensitivity of each cell to calcium, concentration of calcium in the perfusate was increased discontinuously with ratio of 1.8 ranging from 1.0 to 56 mM. When the concentration attained to a threshold, contraction occurred immediately with initial burst followed by slow contraction and completion. Further increase in the concentration did not induce any more contraction. During the initial burst, longitudinal length of the cells became 30—40% of the original size. Fig. 2 shows one case of experiment in which the cell was not responsive to 18 mM or less concentration of calcium and contracted completely by increasing in the concentration at 32 mM $CaCl_2$. The contracted cells could be relaxed when the cells were washed immediately after the contraction by calcium free incubation medium. The relaxed cells were contracted again by subsequent administration of calcium chloride. Calcium contractions of isolated cells could be repeated once or twice but the cells became less sensitive to calcium by repeating the contraction and relaxation.

When isolated cells were incubated with calcium at different concentration, it was observed that every cell did not contract at the same concentration. Fig. 3 represents a histogram showing number of contracted cells at every another 1.8-fold increase in concentration of calcium and relationship between total number of contracted cells and concentration of calcium. When the cells were incubated at various concentration of calcium, the number of contracted cells increased with increase in the concentration at lower concentration of calcium but it decreased at higher concentration. At 10 mM, largest number of cells were contracted.

Time Course of the Calcium Contraction

Time course of the calcium contraction was determined by taking pictures of the contracting cells every 5 or 10 sec and measuring the cell size on prints. When the log length of each contracting cell was plotted against the incubation time, it was appeared that time course of contraction of each cell during the initial burst followed exponential curve. Half time of the contraction could be calculated from the slopes as shown in Table I. Half time at 37° was significantly shorter than that at 30° and the result suggested that the calcium contraction was clearly dependent on temperature.

Effect of Ruthenium Red and 2,4-Dinitrophenol on the Calcium Contraction

Effect of ruthenium red and 2,4-dinitrophenol on the contraction rate was examined. When the cells were contracted by 56 mM calcium at 37° in the presence of 0.01 mM ruthenium red, the half time of the contraction was 24.4±3.1 sec ($n=23$) as shown in Fig. 4 and the value was more than twice of that obtained from control experiment. Incubation with 0.1 mM and

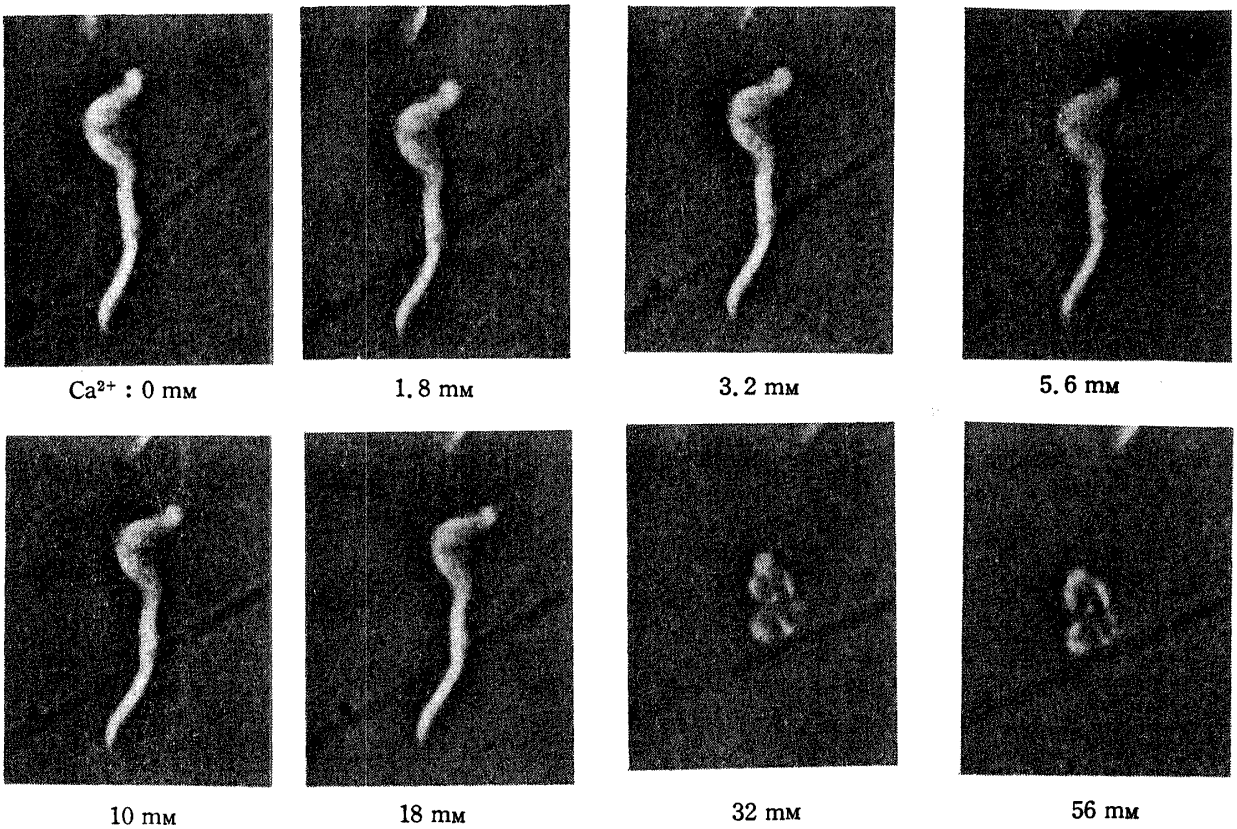

Fig. 2. An Example of Cell Contracted by Increasing Concentration of Calcium Chloride in the Perfusate

Prior to administration of calcium chloride, cell on the dichlorodimethylsilane-coated slide glass were perfused for 60—90 sec with 0.8 ml of the incubation medium. After the preincubation 0.4 ml of the incubation medium containing 1.8 mM $CaCl_2$ was perfused. It took 40—60 sec to complete the perfusion. Just before the perfusion with the 0.4 ml completed, incubation medium containing 3.2 mM $CaCl_2$ was introduced and the perfusion continued. In such manner, concentration of calcium ions in the perfusate increased discontinuously with ratio of 1.8, upto 56 mM. Pictures were taken just before, the following concentration of calcium chloride was administered.

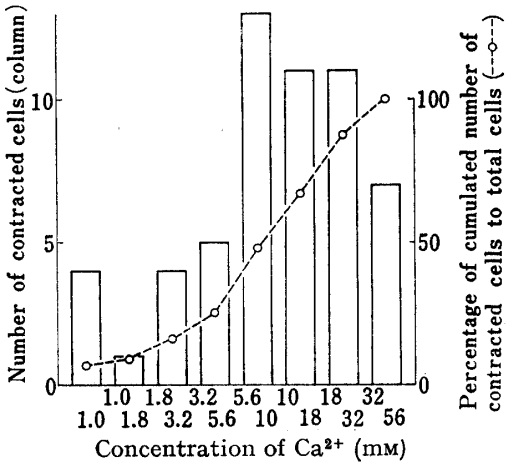

Fig. 3. Contraction of Isolated Cells by Different Concentration of Calcium Chloride

Cells were incubated as described in Fig. 2. Number of contracted cells by 1.8-fold increase in concentration of calcium chloride in the perfusate is expressed as columns and percentage of contracted cells at each concentration of calcium chloride to examined cells ($n=56$) is expressed as broken line.

TABLE I. Half Time of Calcium Contraction of Isolated Cells at Different Temperature

Temperature	Half time for contraction (sec)
30°	25.8±3.3(19)
37°	9.1±1.0(25)

Cells were perfused at 30 or 37° continuously with incubation medium on dichlorodimethylsilane-coated slide glass for 40—60 min and 56.0 mM calcium chloride was administered with the medium. Pictures of the cells were taken at intervals of 5 or 10 sec through the incubation period and size of each cell was determined on prints of the pictures. Half time for the contraction was determined by plotting log size of each cell against incubation time.

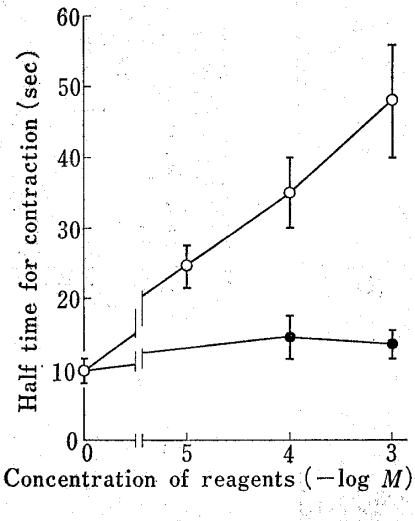

Fig. 4. Effect of Ruthenium Red and 2,4-Dinitrophenol on Calcium Contraction of Isolated Cells

Cells were preliminary incubated with different concentration of the reagent in test tube for 6—15 min at 37° and the cells were placed on dichlorodimethylsilane-coated slide glass. Cells were then perfused continuously with incubation medium containing the same concentration of the reagent for 40—60 sec and 56 mM calcium chloride was administered with the perfusate. Pictures of the contracting cells were taken at intervals of 5 or 10 sec and size of each cell was measured on a prints of the pictures. Half time of the calcium contraction was determined according to procedure as described in Table I. —○— ruthenium red, —●— 2,4-dinitrophenol. Vertical bar represents standard error of mean.

1.0 mM ruthenium red resulted in half times of 34.8±5.2 sec (n=19) and 47.9±7.9 sec(n=24), respectively. The results showed that half time for calcium contraction of isolated cells became longer with increase in concentration of ruthenium red. Although the ruthenium red had been used for staining mucopolysaccharide, the dye did not stain the isolated cells during the incubation.

Effect of 2,4-dinitrophenol on the contraction was also examined and results were shown in Fig. 4. When the cells were incubated with 0.1 mM and 1.0 mM 2,4-dinitrophenol, the half times for the calcium contraction were 13.9±2.7 sec (n=12) and 13.2±1.6 (n=6), respectively. 2,4-Dinitrophenol was not effective on the calcium concentration of isolated cells.

Contraction by Strontium and Barium Ions

Various concentrations of strontium and barium ions were examined for the contraction, instead of the calcium ion. When the cells were exposed to 1.0 mM strontium chloride, several cells showed very rapid contraction followed by spontaneous relaxation. But this phasic contraction did not occur simultaneously in each isolated cell. With 5.0 mM strontium chloride, the rapid contraction also occurred but the spontaneous relaxation was not observed. When a clot, a fragment of the tissue consisted of several cells, was exposed to 5.0 mM strontium chloride, the cells in the clots contracted but each cell did not contract simultaneously. The concentration of 5.0 mM strontium chloride was enough to induce complete contraction of the isolated cells and cells in the clot.

The isolated cells were also contracted by barium ions. When the cells were exposed to 10 mM barium chloride, all the cells contracted very slowly and half time for the contraction was more than 2 min. Perfusion of the cells with lower concentration than 10 mM barium chloride resulted in slower and incomplete contraction.

Discussion

For studies on contraction mechanism of smooth muscle, whole tissue has been used. But, when the whole tissue is used, some effects such as cell to cell interactions, diffusion of agonists or chemicals to the receptor regions, or influence of non-muscular cells to the contraction must be considered. To avoid these problems, introduction of isolated smooth muscle cells might be of use. In the previous paper, the authors made an attempt at use of isolated smooth muscle cells of *vas deferens* of guinea pig to investigate potentiation mechanism of calcium contraction by cocaine and it resulted that cocaine facilitated contraction of individual cells.[1] In the present experiments, some fundamental properties of the calcium contraction were investigated to confirm the previous results.

The present experiments revealed that the isolated cells could be contracted by calcium under the same conditions as the whole tissue was examined, but mode of the contraction was quite different in each others. Calcium contraction of the whole tissue was clearly graded

response as well as it was observed generally with other smooth muscles (Fig. 1). However, when the isolated cells were incubated with increasing in the concentration of calcium, the contraction occurred suddenly at concentration over threshold and it completed without further increase in the concentration (Fig. 2). The contraction looked as if it was all-or-none response while the whole tissue showed graded response. It was demonstrated by the present authors that calcium contraction of isolated smooth muscle cells from taenia coli of guinea pig was graded response when the cells were incubated under the similar conditions as the cells from *vas deferens* were examined.[4] The facts suggested that there might be some significant difference, physiologically, between *vas deferens* and taenia coli. However, the difference of the calcium contraction in isolated cells was remained unclear.

Examination with considerable amount of the isolated cells from *vas deferens* at different concentration of calcium revealed that each isolated cell possessed different sensitivity to calcium (Fig. 3). The curve showing relationship between total number of the contracted cells and concentration of calcium resembled to dose-response curve obtained with the whole tissue (Fig. 1). It may be possible that magnitude of contraction of the muscle is dependent on rate of contracted cells in the tissue.

These isolated cells were not responsive to cholinergic or adrenergic agents while *vas deferens* as a whole tissue could be contracted by both agents. But, the isolated cells proved to be still intact by the facts that these cells were not stained by trypan blue[3] and that the calcium contraction seemed to be all-or-none like response. Since, if the cells were damaged during the isolation and became glycerinated like cells, degree of contraction in each cell must be dependent on concentration of external calcium. Isolated smooth muscle cells from stomach of *Bufo marinus*,[5-14] and from aorta of rabbit[15] were reported to be contracted by the agents. Isolated smooth muscle cells from taenia coli of guinea pig were also contracted by cholinergic agents and degree of the contraction was dependent on concentration of the agents.[16] The fact suggested that smooth muscle of *vas deferens* of guinea pig might be quite different from the smooth muscles of gastrointestinal tract and aorta in respect to resistance of receptor mechanisms against digestion with collagenase.

It was observed in this experiment that the calcium contraction was dependent on temperature (Table I) and rate of the contraction was reduced by ruthenium red, a calcium transport inhibitor[17,18] (Fig. 4). These results suggested that the contraction was not induced merely by changes of osmotic pressure but some physiological mechanisms might participate in the contraction. 2,4-Dinitrophenol was conceivable since the reagent might not act on fundamental mechanisms for contraction of smooth muscle.[19-21]

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Strontium and barium ions could induce contraction of the single cells under the same conditions as calcium ion was examined. Two mechanisms for the contraction could be considered: 1) Those ions acted directly on contractile elements and induced contraction, and 2) the ions led release of tightly bound calcium ion into cytoplasma and the calcium ion acted on the contractile elements. But it was not determined in this experiment whether the strontium and barium ions acted directly or indirectly.

As described above, examination of isolated single cells from smooth muscle might be of use in pharmacological investigations. In the case of *vas deferens*, since the tissue can be contracted by both adrenergic and cholinergic agents, isolation of the chemical transmitter sensitive cells might contribute to further investigations of the muscle.