Characterization of freshly dispersed porcine myometrial cells: evidence for voltage-dependent Ca^{2+} channels and regulatory receptors

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A patch-clamp study of Ca^{2+} currents and spectrofluorometric detection of the intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ was performed on porcine myometrial cells that had been isolated by collagenase. Isolated myometrial cells were the typical long cylinder shape. The main length and diameter of myometrial cells were 505 ± 20 and $11 \pm 0.5 \mu\text{m}$ ($n = 40$), respectively, in the prepartum period and 265 ± 22 and $7 \pm 0.4 \mu\text{m}$ ($n = 40$), respectively, in the luteal phase. Immunocytochemistry with an antibody against desmin stained 90% of the cells positively, and about 95% of the cells excluded Trypan blue dye. The basal $[\text{Ca}^{2+}]_i$ of myometrial cells in the luteal phase and the prepartum period was 119 ± 12 ($n = 30$) and $154 \pm 31 \text{ nmol l}^{-1}$ ($n = 48$), respectively. In prepartum myometrial cells, oxytocin ($10^{-7} \text{ mol l}^{-1}$) and carbachol ($10^{-4} \text{ mol l}^{-1}$) increased $[\text{Ca}^{2+}]_i$ in a biphasic pattern, with a sharp peak followed by a plateau. In cells in the luteal phase, adrenaline ($10^{-7} \text{ mol l}^{-1}$) plus propranolol ($10^{-6} \text{ mol l}^{-1}$) produced a biphasic increase in $[\text{Ca}^{2+}]_i$. However, in the absence of propranolol, the increase in $[\text{Ca}^{2+}]_i$ by adrenaline was small. Prostaglandin $\text{F}_{2\alpha}$ ($10^{-6} \text{ mol l}^{-1}$) induced a monophasic increase in the $[\text{Ca}^{2+}]_i$ in cells in the luteal phase. By depolarizing the cells from -30 to $+50 \text{ mV}$ from a holding potential of -50 mV , Ca^{2+} currents were evoked with a threshold at -20 mV , reaching a maximum between 10 and 30 mV . BayK8644 ($10^{-7} \text{ mol l}^{-1}$), an L-type Ca^{2+} channel agonist, and oxytocin ($10^{-7} \text{ mol l}^{-1}$) enhanced Ca^{2+} currents by $166 \pm 64\%$ and $41 \pm 10\%$, respectively, in prepartum cells. These results suggest that freshly dispersed porcine myometrial cells contain an intact membrane and possess functional voltage-dependent Ca^{2+} channels and receptors for major physiological regulators. Thus, porcine myometrial cells provide a useful model for the study of excitation–contraction coupling and the influence of physiological regulators in the myometrium.

Introduction

Calcium ions play an obligatory role in the coupling between excitation and contraction in smooth muscle. An increase in the intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ by releasing Ca^{2+} from intracellular stores or by the entry of extracellular Ca^{2+} triggers the calmodulin-dependent cascade that induces smooth muscle contraction. Physiologically active agents, such as oxytocin and catecholamine, modulate excitation–contraction coupling by altering $[\text{Ca}^{2+}]_i$ through the release of Ca^{2+} from the sarcoplasmic reticulum or the opening of Ca^{2+} channels in the plasma membrane (Missiaen *et al.*, 1992; Wary, 1993). The critical function of Ca^{2+} in excitation–contraction coupling is demonstrated by the fact that changes in the contractile state of the muscle are often used as a bioassay for changes in $[\text{Ca}^{2+}]_i$ (Minneman, 1988). However, it is difficult to explore the

cellular mechanisms of excitation–contraction coupling and its modulation by different agents *in vivo*, or even in intact muscle strips, with respect to Ca^{2+} . The use of isolated single smooth muscle cells offers several advantages over the multicellular smooth muscle preparation, because single smooth muscle cells (i) lack a dense connective tissue matrix that may serve as a diffusion barrier to agents (Toro *et al.*, 1986; Warshaw *et al.*, 1986), (ii) lack a synaptic terminal and varicosities that may contribute to the release of neurotransmitters during extracellular stimulations (Amédée *et al.*, 1986), (iii) lack gap junctions that form a functional syncytium (Garfield and Cole, 1989), complicating intracellular signal determination, and (iv) can be used for patch-clamp (Hamill *et al.*, 1981) and spectrofluorometric Ca^{2+} determination techniques, both of which have expanded our understanding of the mechanism of contraction and its modulations in smooth muscle cells.

Several reports describe the isolation of myometrial cells and the characterization of electrophysiological and biochemical properties of freshly dispersed or cultured myometrial cells (Casey *et al.*, 1984; Amédée *et al.*, 1986; Boulet and Fortier,

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1987; Kyojuka *et al.*, 1987; Pressman *et al.*, 1988; Ohya and Sperelakis, 1989; Phillippe *et al.*, 1990). However, most of these studies were performed in rodents. There is increasing evidence that excitation–contraction coupling and uterine functions are species specific. For example, α_1 - but not α_2 -adrenoceptors mediate an increase in myometrial contractions in rats (Digges, 1982), whereas in ewes (Marnet *et al.*, 1987; Prud'Homme, 1988) and sows (Ko *et al.*, 1990; Yang and Hsu, 1993), myometrial contractions are mediated by α_2 - but not α_1 -adrenoceptors.

The primary objective of this study was to develop a freshly dispersed porcine myometrial cell model with functional voltage-dependent Ca^{2+} channels and receptors having intact signal transduction pathways. With this model, the modulation of myometrium contractility was assessed by hormonal and neural agents, using Ca^{2+} as a signal.

Materials and Methods

Isolation of porcine myometrial cells

Porcine uteri were obtained from a local abattoir and a surgical laboratory. Uteri at either late (days 107–112) pregnancy or the luteal phase of the oestrous cycle were used, because porcine myometrium at these stages are known to respond well to pharmacological contractants (Yang and Hsu, 1993; Yu *et al.*, 1993). The pregnancy status of the uterus was determined by the method of Evans and Sack (1973); the luteal phase was determined by visually inspecting the ovary containing corpora lutea, which at this stage were dark red to wine red (Arthur *et al.*, 1989).

The mid-portion of the uterine horn was dissected and placed into Ca^{2+} - and Mg^{2+} -free Hank's balanced salt solution (HBSS) containing 137 mmol NaCl l^{-1} , 0.66 mmol KH_2PO_4 l^{-1} , 5.4 mmol KCl l^{-1} , 0.35 mmol Na_2HPO_4 l^{-1} , 4.2 mmol dextrose l^{-1} , 10 mmol Hepes l^{-1} and 11 mg phenol red l^{-1} (pH 7.4). After being transported to the laboratory, the tissue was rinsed with HBSS in a Petri dish and longitudinal muscle segments were excised. The segments were further minced into approximately 2 mm $\times$ 1 mm pieces and washed twice with HBSS. The minced tissue was placed in a 25 ml spinner flask (Bellco Glass, Vineland, NJ) and incubated in HBSS containing 0.1% collagenase (352 U mg^{-1} , CLS II, Worthington Biochemical, Freehold, NJ), and 0.2% BSA at 37°C. The tissue segments were stirred continuously at a rate of approximately 60 cycles min^{-1} to keep the undigested segments suspended. After the incubation with collagenase for 60 min and 20 min, respectively, the supernatant was discarded, because it contained debris, round stroma and endothelial cells. Fresh enzyme solution was added to the tissue suspension every 20 min, and the supernatant was collected and examined for the presence of myometrial cells, until the required amounts of cells were harvested. The cells were washed and centrifuged at 50 g, 25°C for 10 min, and resuspended in a modified Krebs-Ringer bicarbonate solution containing 118 mmol NaCl l^{-1} , 1.25 mmol CaCl_2 l^{-1} , 1.19 mmol KH_2PO_4 l^{-1} , 4.74 mmol KCl l^{-1} , 0.5 mmol MgSO_4 l^{-1} , 8 mmol dextrose l^{-1} , 10 mmol Hepes l^{-1} and 0.1% BSA (pH 7.4) for $[\text{Ca}^{2+}]_i$ determinations.

To obtain cells with minimal contractions for recording the Ca^{2+} current in whole cells, a modification of the above protocol was used. The tissue segments were placed in a 20 ml scintillation vial with HBSS containing 0.5 mmol MgCl_2 l^{-1} , 0.1% collagenase, 0.1% BSA and 0.02% trypsin inhibitor (Sigma Chemical Co., St Louis, MO), and stirred gently at 37°C for approximately 40 min. The supernatant was discarded and the pellet was transferred to HBSS. The pellet was then gently triturated with a fire-polished plastic pipette and the cell suspension was placed in a culture dish with coverslips (3 mm $\times$ 3 mm) in the Krebs-Ringer bicarbonate solution and kept at 4°C until use. The cells usually attached to the coverslip within 15 min and were used on the same day. Only the cells with a bright and smooth appearance were used.

Immunocytochemistry

Cells were obtained under sterile conditions from sows on day 112 of pregnancy using the method described above, and were seeded into Petri dishes with histological slides that had previously been coated with poly-L-lysine. Waymouth MB752/1 medium (Sigma Chemical Co.) plus 10% fetal bovine serum and 50 μg gentamicin ml^{-1} was used for cell culture. The cells were incubated at 37°C under an atmosphere of 5% CO_2 and 95% air, fixed with 4% paraformaldehyde after 2 days, and stained for desmin by a modification of the avidin–biotin complex method (Hsu *et al.*, 1981). Endogenous peroxidase activity was inhibited by incubating the cells in 0.3% H_2O_2 for 30 min. After incubating the cells for 2 h with normal goat serum, they were incubated in primary anti-rabbit desmin monoclonal antibody (Sigma Chemical Co.) at a dilution of 1:100 for 24 h at room temperature. The secondary biotinylated anti-IgG serum (Vector Laboratories, Burlingame, CA) was applied for 2 h at room temperature, followed by an incubation with the avidin–biotin–horseradish-peroxidase complex. The cells were washed between incubations with a solution containing 154 mmol NaCl l^{-1} , 43 mmol KH_2PO_4 l^{-1} and 23 mmol KHPO_4 l^{-1} . Immunologically bound peroxidase was visualized after incubating the cells for 18 min in 0.1 mol sodium acetate l^{-1} containing 0.4 mg diaminobenzidine ml^{-1} and 0.05% H_2O_2 . Finally, the cells were lightly counterstained with neutral red, dehydrated and mounted.

Determination of $[\text{Ca}^{2+}]_i$

The cells (10^6 ml^{-1}) were incubated with 4 μmol Fura-2-acetoxymethylester l^{-1} (Molecular Probes, Eugene, OR) for 45 min at 37°C in Krebs-Ringer bicarbonate solution with gentle shaking. The cell mixture was then centrifuged at 50 g for 10 min, and resuspended in Krebs-Ringer bicarbonate solution (2×10^5 ml^{-1}) for at least 20 min before determining the $[\text{Ca}^{2+}]_i$ using a SLM8000 spectrofluorometer (SLM Instruments, Urbana, IL), as described by Hsu *et al.* (1991).

Ca^{2+} current recordings

The whole-cell currents were recorded as described by Hamill *et al.* (1981) using an Axopatch-1D clamp amplifier (Axon instruments, Foster City, CA). Patch pipettes (3–5 M Ω)

were made on a micropipette puller (Narishige Instruments, Tokyo) using disposable glass pipettes (VWR Scientific, West Chester, PA), and were polished on a custom-made microforge. A high-resistance seal between the edge of the electrode and the membrane was obtained by applying slight suction to the pipette after gently pushing it on to the surface of the cell, which was viewed on a TV monitor. The liquid junction potentials were nullified with an offset circuit before the high-resistance seal formed. Access to the intracellular milieu was obtained by applying a negative pressure to the pipette. Ca²⁺ currents were evoked by step depolarizations lasting 300 ms every 20 s from a holding potential of -50 mV, and with a first step from -30 mV followed by subsequent steps at 10 mV increments. Linear resistance and capacitive currents were electronically compensated by a P/N protocol with $n = 4$. Data were collected and analysed using an IBM-PC computer with an analogue-digital interface board and Pclamp software 5.1.1 (Axon instruments, Foster City, CA). The patch pipette was filled with a solution containing 125 mmol Cs glutamate l⁻¹, 10 mmol CsCl l⁻¹, 2 mmol Mg-ATP l⁻¹, 10 mmol Na₂EGTA l⁻¹ and 10 mmol Hepes l⁻¹ (pH 7.2). The bath solution contained 125 mmol choline chloride l⁻¹, 20 mmol tetraethylammonium bromide l⁻¹, 5 mmol 4-aminopyridine l⁻¹, 2 mmol BaCl₂ l⁻¹, 10 mmol dextrose l⁻¹ and 10 mmol Hepes l⁻¹ (pH 7.3). Agents were applied to the bath solution directly and all experiments were performed at room temperature.

Drug preparations

Adrenaline bitartrate, carbachol chloride, oxytocin and propranolol HCl were purchased from Sigma Chemical Co. Prostaglandin F_{2α}, tromethamine and BayK8644 were purchased from Upjohn (Kalamazoo, MI) and Research Biochemical International (Natick, MA), respectively. Carbachol and propranolol were dissolved in distilled water. Adrenaline and oxytocin were prepared in 0.1% ascorbic acid and Krebs-Ringer bicarbonate, respectively. BayK8644 was dissolved in anhydrous ethanol.

Statistical analyses

All values are expressed as means ± SEM. Data were analysed by Student's paired *t* test. The significance level was set at $P < 0.05$.

Results

Morphology of porcine myometrial cells

After dispersion, porcine myometrial cells appeared as long and thin cylinders under inverted microscopy (Fig. 1). The length and diameter of prepartum cells were $505 \pm 20 \mu\text{m}$ and $11 \pm 0.5 \mu\text{m}$ ($n = 40$), respectively, while in cells at the luteal phase these measurements were $265 \pm 22 \mu\text{m}$ and $7 \pm 0.4 \mu\text{m}$ ($n = 40$), respectively. When the cell isolation protocol for the Ca²⁺ current recordings was used, the smooth appearance indicated that most cells were in their relaxed state (Fig. 1a).

Fig. 1. Light phase-contrast micrographs of freshly dispersed smooth muscle cells from the prepartum porcine uterus. (a) Relaxed smooth muscle cells for the patch-clamp study. (b) Variably contracted smooth muscle cells for determination of the intracellular Ca²⁺ concentration. Scale bars represent 125 μm .

However, during the Ca²⁺ determination procedure many cells became contracted, with a rippled surface and shorter (Fig. 1b), compared with the relaxed cells for the patch-clamp technique.

Verification and viability of porcine myometrial cells

Myometrial cells can be identified morphologically. Desmin, however, is a specific marker found in the cytoskeleton of muscle cells (Lazarides, 1982). In this study, 90% freshly dispersed cells stained positively with anti-desmin immunocytochemistry after culture for 2 days (Fig. 2), whereas negative controls failed to stain with the anti-desmin monoclonal antibody (data not shown). These results confirmed that the freshly dispersed cells were smooth muscle cells. Compared with freshly dispersed cells, the cells were slightly more rounded and shorter after 2 days in culture. The desmin granules were seen in the cytosol of these cells. Almost all (95%) of the myometrial cells excluded Trypan blue, indicating that they were viable with an intact cell membrane. At least 90% cells appeared bright under the

Fig. 2. Light micrograph of prepartum myometrial cells after 2 days in culture stained with anti-desmin monoclonal antibody using immunocytochemistry. Scale bar represents 125 μm .

microscope, which is a further indication of their viability (Pressman *et al.*, 1988).

Responses of $[\text{Ca}^{2+}]_i$ to agonists

To determine whether the isolated porcine myometrial cells were functioning, the $[\text{Ca}^{2+}]_i$ was determined and the Ca^{2+} current was recorded. The mean resting $[\text{Ca}^{2+}]_i$ of freshly dispersed cells from uteri in the luteal phase and the prepartum period was 119 ± 12 ($n = 30$) and 154 ± 31 nmol l^{-1} ($n = 48$), respectively, using Fura-2 as the Ca^{2+} indicator. Oxytocin and carbachol caused an increase in $[\text{Ca}^{2+}]_i$ of prepartum cells in the presence of an extracellular Ca^{2+} concentration of 1.25 mmol l^{-1} (Fig. 3). Oxytocin (10^{-7} mol l^{-1}) significantly increased $[\text{Ca}^{2+}]_i$ from a basal value of 153 ± 14 nmol l^{-1} to a peak of 244 ± 15 nmol l^{-1} , followed by a plateau concentration (192 ± 12 nmol l^{-1} , taken at 3 min after oxytocin application; $n = 9$) (Fig. 3a). Carbachol (10^{-4} mol l^{-1}) also significantly increased $[\text{Ca}^{2+}]_i$ from a basal value of 156 ± 15 nmol l^{-1} to a peak of 256 ± 10 nmol l^{-1} , and a plateau of 201 ± 13 nmol l^{-1} ($n = 4$) (Fig. 3b).

Adrenaline in the presence and absence of propranolol and $\text{PGF}_{2\alpha}$ caused an increase in $[\text{Ca}^{2+}]_i$ of cells at the luteal stage in the presence of an extracellular Ca^{2+} concentration of 1.25 mmol l^{-1} (Fig. 4). After pretreatment with propranolol (10^{-6} mol l^{-1}) for 5 min, adrenaline (10^{-7} mol l^{-1}) significantly increased $[\text{Ca}^{2+}]_i$ from 120 ± 10 nmol l^{-1} to 195 ± 8 nmol l^{-1} , and this concentration was then maintained at a plateau of 154 ± 14 nmol l^{-1} ($n = 5$) (Fig. 4a). In the absence of propranolol (10^{-6} mol l^{-1}), adrenaline (10^{-7} mol l^{-1}) only slightly but significantly increased $[\text{Ca}^{2+}]_i$ from a basal value of 122 ± 6 to 148 ± 7 nmol l^{-1} ($n = 5$) (Fig. 4b). Prostaglandin $\text{F}_{2\alpha}$ (10^{-6} mol l^{-1}) caused a monophasic increase of $[\text{Ca}^{2+}]_i$ from a basal value of 119 ± 11 to 150 ± 8 nmol l^{-1} in cells at the luteal phase ($P < 0.05$, $n = 3$) (Fig. 4c). The responses of $[\text{Ca}^{2+}]_i$ to oxytocin and carbachol were similar in the luteal phase and prepartum cells (data not shown).

Fig. 3. Effects of (a) 10^{-7} $\text{mol oxytocin l}^{-1}$ and (b) 10^{-4} $\text{mol carbachol l}^{-1}$ on the intracellular Ca^{2+} concentration of freshly dispersed smooth muscle cells from prepartum porcine myometrium. Arrowheads show the times when the agents were added.

Properties of voltage-dependent Ca^{2+} channels and the influence of BayK8644 and oxytocin

After blocking K^{+} currents by adding a high concentration of Cs^{+} (125 mmol l^{-1}) to the pipette solution, and tetraethylammonium and 4-aminopyridine to the bath solution, an inward current (Ba^{2+} current; I_{Ba}) was elicited by depolarizing from -30 to $+50$ mV from a holding potential of -50 mV (Fig. 5a, Fig. 6a) in the presence of 2 $\text{mmol Ba}^{2+} \text{l}^{-1}$ in the bath solution. The currents were activated at the membrane potential of -20 mV and reached a maximum between 10 and 30 mV . The maximal amplitude was approximately

Fig. 4. Effects of (a, b) 10^{-7} mol l^{-1} and (c) 10^{-6} mol $\text{PGF}_{2\alpha}$ l^{-1} on the intracellular concentration of Ca^{2+} in freshly dispersed smooth muscle cells from a porcine myometrium in the luteal phase. Arrowheads show the times when the agents were added. Note that in (a) cells were pretreated with 10^{-6} mol propranolol l^{-1} for 5 min before adrenaline was applied.

100–500 pA ($n = 12$) in the cells at the luteal phase and 200–1500 pA ($n = 20$) in the prepartum cells. In the prepartum cells, BayK8644 (10^{-7} mol l^{-1}) increased the peak amplitude by $166 \pm 64\%$ ($n = 6$, $P < 0.05$, compared with the control tests) (Fig. 5). The I–V curve shifted to the left after administering BayK8644 although the shape of the curve was maintained. The Ca^{2+} current recovered after the washout. Oxytocin (10^{-7} mol l^{-1}) increased the peak amplitude by $41 \pm 10\%$ ($n = 7$, $P < 0.05$, compared with the control tests) after 3 min of application (Fig. 6). The peak potential was not affected by oxytocin treatment. The Ca^{2+} currents also recovered after the washout.

Discussion

We described a method for isolating myometrial cells from porcine uteri during the luteal phase of the oestrous cycle and preparturition that can be manipulated as a population or as single cells. The use of cultured cells appears to be an attractive solution for overcoming the limitations of the intact muscle preparation. However, slight variations in the isolation method and culture process may alter the properties of the cultured cells (Kocher *et al.*, 1984; Boulet and Fortier, 1987). The changes in the properties of the cultured myometrial cells may be partially attributed to the lack of sex steroids that regulate uterine function *in vivo*. In fact, if sex steroids are applied to cultured human myometrial cells they remain responsive to contractants for up to 7 days (Pressman *et al.*, 1988), whereas without hormonal application rat myometrial cells lose their responsiveness to contractants within 36 h (Amédée *et al.*, 1986).

The long and thin cylinder features observed in our isolated porcine myometrial cells appear to be very similar to those of the rat (Koyozuka *et al.*, 1987) and human (Pressman *et al.*, 1988) isolated myometrial cells and other smooth muscle cells

(Warshaw *et al.*, 1986) visualized under light microscopy. The granular distribution of desmin is also consistent with the findings of Boulet and Fortier (1987), who observed that 90% of rabbit myometrial cells have desmin granules after culture for 4 days. The myometrial cells were easily identified by their morphology. Immunocytochemistry, however, was useful for verifying the viability of the cells and also determining the percentage of smooth muscle cells in a population (Loeb *et al.*, 1985), which is critical in determining the $[\text{Ca}^{2+}]_i$ in a population, as cells other than myometrial cells may have a different response to contractants.

A major problem in the interpretation of such a study is that the dispersion process could damage functional units, such as ion channels and receptors. In our study, we conclude that the freshly dispersed porcine myometrial cells were only minimally damaged by enzymes for the following reasons: 95% of the cells excluded Trypan blue and had a bright appearance; there was a low extent of Ca^{2+} indicator leakage, as indicated by a stable fluorescent ratio of 340:380; and satisfactory responses to stimulants were obtained.

Because both electromechanical and pharmacomechanical coupling are important in excitation–contraction coupling, voltage-dependent Ca^{2+} channels and major contractant receptors were evaluated in the freshly dispersed porcine myometrial cells. Voltage-dependent Ca^{2+} channels are responsible for the upstroke of action potential to induce and maintain the long-term myometrial contraction by mediating the entry of Ca^{2+} from the external environment (Missiaen *et al.*, 1992). The voltage-dependent Ca^{2+} channels in the murine and human myometrial cells are mainly L-type (Ohya and Sperelakis, 1989; Young *et al.*, 1993), although a few T-type Ca^{2+} channels have also been identified (Young *et al.*, 1993). The fact that Ca^{2+} currents are activated by depolarization and administration of BayK8644 suggests that there are L-type Ca^{2+} channels in the porcine myometrium. The voltage-dependent Ca^{2+} channel is activated not only by membrane depolarization but also by hormonal and neural stimulations. The voltage-dependent Ca^{2+} channel blocker verapamil greatly inhibits oxytocin-induced porcine myometrial contractions (Yu *et al.*, 1993), supporting our findings that oxytocin stimulates the contractions by partly increasing $[\text{Ca}^{2+}]_i$ through opening these channels. However, our results contradict those obtained from rat myometrial cells, in which oxytocin was found to inhibit Ca^{2+} currents (Inoue *et al.*, 1992).

Myometrial contractility is spontaneously controlled by electrical activity *in vivo* and is highly regulated by neural and hormonal factors. Of the hormonal regulation, oxytocin and $\text{PGF}_{2\alpha}$ are two potent stimulators for myometrial contractions. Several lines of evidence suggest that both of them induce contractions by increasing $[\text{Ca}^{2+}]_i$ (Anwer and Sanborn, 1989; Mackenzie *et al.*, 1990; Molnar and Hertelendy, 1990; Tasaka *et al.*, 1991). The present results suggest that oxytocin and $\text{PGF}_{2\alpha}$ receptors and their signal transduction pathways are intact in the freshly dispersed porcine myometrial cells, because activation of these receptors by agonists increases $[\text{Ca}^{2+}]_i$. With regards to neural modulation, adrenergic and cholinergic nerves have a great influence on myometrial contractility. Activation of α -adrenoceptors and cholinergic receptors increases contractions, whereas activation of β -adrenoceptors promotes relaxation (Wary, 1993). Carbachol and adrenaline in

Fig. 5. Effect of BayK8644 on the inward Ba^{2+} current (I_{Ba}) through Ca^{2+} channels in prepartum porcine myometrial cells. (a) (i) Control I_{Ba} elicited by 300 ms depolarization steps from -30 mV to $+50$ mV from a holding potential of -50 mV in the absence of BayK8644. (ii) I_{Ba} elicited by the same depolarization protocol in the presence of 10^{-7} mol BayK8644 l^{-1} . (iii) The I_{Ba} tracings obtained 5 min after the washout of BayK8644. (b) Corresponding I-V curve of peak I_{Ba} before (Δ) and after ($\blacktriangle$) the application and washout ($\diamond$) of BayK8644.

Fig. 6. Effect of oxytocin on the inward Ba^{2+} current (I_{Ba}) through Ca^{2+} channels in prepartum porcine myometrial cells. (a) (i) Control I_{Ba} tracings elicited by 300 ms depolarization steps from -30 mV to $+50$ mV from a holding potential of -50 mV in the absence of oxytocin. (ii) The I_{Ba} tracings obtained by the same depolarization protocol 3 min after the application of 10^{-7} mol oxytocin l^{-1} . (iii) The I_{Ba} tracings obtained 5 min after the washout of oxytocin. (b) The effect of oxytocin and its recovery on the I-V curve of peak I_{Ba} : ($\circ$) before; ($\bullet$) after oxytocin application and (∇) washout.

the presence of a β -blocker propranolol increases $[\text{Ca}^{2+}]_i$, suggesting that cholinergic, α - and β -adrenoceptors were intact in this preparation.

Little is known about the basic characteristics of excitation-contraction coupling in the myometrium; information about Ca^{2+} stores and how they release Ca^{2+} , Ca^{2+} channels and Ca^{2+} entry is scarce. This prevents assessment of their regulation by

physiological agents. In fact, even for the recognized major regulators of myometrial contraction, such as prostaglandins and oxytocin, surprisingly little is known about their mechanism of action at the cellular level (Parkington and Coleman, 1990). However, the patch-clamp technique and $[\text{Ca}^{2+}]_i$ determination have been very useful in addressing the mechanism of excitation-contraction coupling and its regulation in cardiac

and skeletal muscles, and other smooth muscle cells. These techniques should facilitate the study of mechanisms of excitation–contraction coupling and their regulation in the myometrium, particularly in higher mammals.

In conclusion, we have developed a method to obtain freshly dispersed porcine myometrial cells with an integral structure. These cells express functional voltage-dependent Ca²⁺ channels and receptors for major contractants. They should be useful in the study of mechanisms of excitation–contraction coupling and their modulation by hormonal and neuronal agents with biochemical and electrophysiological approaches.

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