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BASIC RESEARCH

Sources of calcium in agonist-induced contraction of rat distal colon smooth muscle *in vitro*

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Abstract

AIM: To study the origin of calcium necessary for agonist-induced contraction of the distal colon in rats.

METHODS: The change in intracellular calcium concentration ($[Ca^{2+}]_i$) evoked by elevating external Ca^{2+} was detected by fura 2/AM fluorescence. Contractile activity was measured with a force displacement transducer. Tension was continuously monitored and recorded using a Powerlab 4/25T data acquisition system with an ML110 bridge bioelectric physiographic amplifier.

RESULTS: Store depletion induced Ca^{2+} influx had an effect on $[Ca^{2+}]_i$. In nominally Ca^{2+}-free medium, the sarco-endoplasmic reticulum Ca^{2+}-ATPase inhibitor thapsigargin (1 μmol/L) increased $[Ca^{2+}]_i$ from 68 to 241 nmol/L, and to 458 ($P < 0.01$) and 1006 nmol/L ($P < 0.01$), respectively, when 1.5 mmol/L and 3.0 mmol/L extracellular Ca^{2+} was reintroduced. Furthermore, the change in $[Ca^{2+}]_i$ was observed with verapamil (5 μmol/L), La^{3+} (1 mmol/L) or KCl (40 mmol/L) in the bathing solution. These channels were sensitive to La^{3+} ($P < 0.01$), insensitive to verapamil, and voltage independent. In isolated distal colons we found that in normal Krebs solution, contraction induced by acetylcholine (ACh) was partially inhibited by verapamil, and the inhibitory rate was 41% ($P < 0.05$). On the other hand, in Ca^{2+}-free Krebs solution, ACh induced transient contraction due to Ca^{2+} release from the intracellular stores. The transient contraction lasted until the Ca^{2+} store was depleted. Restoration of extracellular Ca^{2+} in the presence of atropine produced contraction, mainly due to Ca^{2+} influx. Such contraction was not inhibited by verapamil, but was decreased by La^{3+} (50 μmol/L) from 0.96 to 0.72 g ($P < 0.01$).

CONCLUSION: The predominant source of activator Ca^{2+} for the contractile response to agonist is extracellular Ca^{2+}, and intracellular Ca^{2+} has little role to play in mediating excitation-contraction coupling by agonists in rat distal colon smooth muscle *in vitro*. The influx of extracellular Ca^{2+} is mainly mediated through voltage-, receptor- and store-operated Ca^{2+} channels, which can be used as an alternative to develop new drugs targeted on the dysfunction of digestive tract motility.

Key words: Distal colon; Source of Ca^{2+}; Store-operated Ca^{2+} channel; Voltage-operated Ca^{2+} channel; Acetylcholine

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INTRODUCTION

Ca^{2+} is involved in many cellular transduction pathways, and regulation of different cellular phenotypes by $[Ca^{2+}]_i$ has been observed in many cell systems, including smooth muscle cells (SMC). Various agonists induce contraction in a variety of smooth muscle preparations, with a concomitant increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)[1,2]. The source for this ion is extracellular Ca^{2+} influx and Ca^{2+} release from intracellular stores. The relative contribution of intracellular and extracellular sources of Ca^{2+} depends on the tissue and mode of stimulation[3]. Moreover, agonist-induced increases in the cytosolic Ca^{2+} concentration produce smooth muscle contraction, characterized by a fast initial peak, the phasic component, followed by a decline to a lower maintained tension level, the tonic component[4,5].

In the former, we think two different Ca^{2+} channels participate in extracellular Ca^{2+} influx: voltage-operated Ca^{2+} channels (VOCCs) and receptor-operated Ca^{2+} channels (ROCCs)[6,7]. Participation of these channels in contraction varies among the types of smooth muscle present, the

agonists used, and the experimental conditions planned.

In addition to these influx pathways, there is now significant data from different cell types that demonstrate controlled Ca^{2+} influx also occurs in response to depletion of intracellular Ca^{2+} stores. We call these store-operated Ca^{2+} channels (SOCCs) or capacitative Ca^{2+} entry (CCE) channels. Previous studies have suggested SOCCs are restricted in non-excitable cells[8,9], but they have only recently been investigated in excitable cells , such as smooth muscle cells and neurons, which are involved in smooth muscle tone and regulation of synaptic plasticity. It has been reported there is a relationship between CCE and the regulation of smooth muscle tone with respect to their involvement in pulmonary, stomach, bronchial, vascular, gallbladder, and basilar artery tissue[10-15]. There is thus far no data to show that CCE are necessary as a source of Ca^{2+} in the smooth muscle of the rat distal colon.

The neurotransmitter acetylcholine (ACh) induces smooth muscle contraction *via* activating muscarinic receptors. ACh has been shown to elicit contraction that consists of a rapid phasic phase, followed by a tonic phase, by activation of M_3 muscarinic receptors in the rat and guinea pig gastric fundus and ileum, and the human ileum[16-19]. However, there have been some different results concerning the source of Ca^{2+} for muscarinic receptor-mediated contraction. It has been postulated that the sources of Ca^{2+} for phasic and tonic contraction induced by activation of muscarinic receptors differ among regions of the digestive tract and species of animals used.

It has been suggested that pharmacological manipulation of Ca^{2+} channels may be therapeutically useful in treating colonic motility disorders. Therefore, we tried to clarify the source of Ca^{2+} in the contraction induced by ACh. Sources of activator Ca^{2+} for muscarinic-mediated contraction of proximal colonic smooth muscle in rats have been well documented. With respect to the rat distal colon, the relative importance of the contribution of the different Ca^{2+} influx mechanisms, and the release of Ca^{2+} from the sarcoplasmic reticulum (SR), has not been assessed. The aim of this study was to evaluate the contribution of VOCCs, non-VOCCs and intracellular Ca^{2+} release to ACh-induced contraction in the smooth muscle of rat distal colon *in vitro*.

MATERIALS AND METHODS

Tissue preparation

A segment of distal colon (about 2 cm) was removed from Sprague-Dawley rats (150-250 g, supplied by the animal center of Anhui Medical University, China) killed by cervical dislocation. Pieces of colon were cleaned in a Petri dish, by perfusing with Krebs solution (114.0 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 2.5 mmol/L $CaCl_2$, 1.8 mmol/L NaH_2PO_4, 11.5 mmol/L glucose, 18.0 mmol/L $NaHCO_3$, pH 7.4). The adipose and connective tissues were carefully stripped off with fine forceps. The endothelium of the colon was removed by gently rubbing the inner lumen of the vessels with a rough wooden stick.

Tension measurement

Pieces of distal colon were mounted in 40-mL organ baths containing Krebs solution, maintained at 37℃ and gassed with 95% O_2 and 50% CO_2, pH 7.4. One end of each piece was fixed to a hook on the bottom of the bath and the other attached to a force displacement transducer (Xinhang, Beijing, China). A resting preload of 1 g was applied to each colon, which was then allowed to equilibrate for 1 h. During this time, the Krebs solution was changed every 20 min. Tension was continuously monitored and recorded using a Powerlab 4/25T data acquisition system (Australia AD Instruments) with an ML110 bridge bioelectric physiographic amplifier (Australia AD Instruments). ACh and atropine were dissolved in the perfusate on the day of use.

Cell preparation

A segment of the distal colon was removed and placed in oxygenated HEPES/Ringer buffer solution (126 mmol/L NaCl, 6 mmol/L KCl, 6 mmol/L HEPES, 11 mmol/L $MgCl_2$, 1.5 mmol/L $CaCl_2$), pH 7.4 with 5 mol/L NaOH. The fecal contents were removed by repeated rinsing with HEPES/Ringer solution. After the segment was cut open longitudinally and pinned out in a dissection dish. Under a microscope, longitudinal and circular muscle layers were freed of the mesentery, serosa and mucosa, and cut into small pieces (2 mm^2) and rinsed in HEPES/Ringer solution for 20 min (at 37℃). Dispersion of smooth muscle cells was accomplished by three incubations at 31℃ with agitation in low-Ca^{2+} HEPES/Ringer solution containing collagenase type I (0.6 mg/mL), bovine serum albumin (2 mg/mL) and soybean trypsin inhibitor (0.6 mg/mL). They were then suspended in enzyme-free low-Ca^{2+} HEPES/Ringer solution and gently agitated through the tip of a wide-bore glass pipette to liberate single smooth muscle cells. Isolated cells were collected by low-speed centrifugation and resuspended in HEPES/Ringer solution. Trypan blue exclusion was used to verify cell viability (> 90% of all cells).

Measurement of $[Ca^{2+}]_i$ in distal colon smooth muscle cells

Measurement of $[Ca^{2+}]_i$ in colonic smooth muscle cells using fura-2 was performed using dual excitation wavelength fluorescence microscopy. Aliquots of cell suspension in HEPES/Ringer solution (10^6 cells/mL) were placed in Quartz cuvettes and incubated with 5 μmol/L fura-2/AM at 37℃ for 45 min. After being loaded with fura-2/AM, the cells were rinsed with Ca^{2+}-free solution, centrifuged (1500 r/min) and suspended in Ca^{2+}-free solution for the experiments that were carried out in a 960 MC spectrofluorophotometer (Shanghai Precision & Scientific Instruments, Shanghai, China). Cells were alternately illuminated at 340 and 380 nm and the intensity of fluorescence emission was measured at 500 nm. The $[Ca^{2+}]_i$ was calculated according to Grynkiewicz's equation: $[Ca^{2+}]_i = Kd\ [(F - F_{min})/(F_{max} - F)]$: where the dissociation constant Kd was 224 nmol/L at 37℃; F was fluorescence intensity under different experiment conditions; F_{max} was the maximum fluorescence intensity obtained in the presence of 0.1% Triton ×-100; and the minimum value (F_{min}) was obtained by using 5 mmol/L EGTA in the external medium. Before calculation, the autofluorescence measured from cells that unloaded

Table 1 Measurement of $[Ca^{2+}]_i$ in freshly dispersed suspension of smooth muscle cells from rat distal colon

Group	$[Ca^{2+}]_i$ (nmol/L)		
	Ca^{2+}-free medium	1.5 mmol/L $CaCl_2$ medium	3.0 mmol/L $CaCl_2$ medium
Control	68.32 ± 3.43	104.81 ± 6.37	194.44 ± 3.91
Thapsigargin	240.85 ± 12.65	457.55 ± 19.80[b]	1005.93 ± 54.62[b]

[b]$P < 0.01$ *vs* control.

fura-2/AM should be subtracted. All the measurements were finished in 40 min.

Drugs and solutions

Reagents were procured from the following vendors: thapsigargin, DMSO, ACh, atropine, lanthanum, fura-2/AM, Triton ×-100, EGTA, collagenase type I , bovine serum albumin (BSA) and verapamil were from Sigma (USA), and soybean trypsin inhibitor was from GIBCO (USA). The solutions of thapsigargin and fura-2/AM were prepared by dilution with DMSO. The concentration of DMSO in the final solution was < 0.1% and did not affect $[Ca^{2+}]_i$. In cell experiments, the Ca^{2+}-free HEPES/Ringer solution was prepared without $CaCl_2$ and with addition of EGTA (0.2 mmol/L). The low-Ca^{2+} solution had the same composition, except $CaCl_2$ was 0.03 mmol/L.

Statistical analysis

Biostatistical analysis was performed using the SPSS 11.0 software package. All experiments were repeated at least three times. Results of multiple experiments are given as the mean ± SE. Unpaired Student's *t* test was used for statistical evaluation in two-group comparisons. $P < 0.05$ was accepted as statistically significant.

RESULTS

Establishment of SOCCs

We first established whether SOCCs were present in rat distal colon smooth muscle cells. As shown in Table 1, for the fura-2/AM loaded cells, the incubation in Ca^{2+} free solution lasted 3 min, followed by the addition of two concentrations of Ca^{2+} (1.5, 3.0 mmol/L). It can be seen that cytosolic calcium rose from 68.32 in Ca^{2+}-free solution to 104.81 and 194.44 nmol/L for the two concentrations of 1.5 mmol/L and 3.0 mmol/L, respectively. The incubation of cells in Ca^{2+}-free solution in the presence of thapsigargin (1 μmol/L), followed by the addition of Ca^{2+}, 1.5 and 3.0 mmol/L, increased $[Ca^{2+}]_i$ that was significantly greater (455.77 and 1005.9 nmol/L) than that in the absence of thapsigargin.

Sensitivity to verapamil

To distinguish SOCC-mediated Ca^{2+} influx from that *via* L-type Ca^{2+} channels, we conducted experiments in a separate set of cells using 5 μmol/L verapamil. In these cells pretreated with verapamil, the $[Ca^{2+}]_i$ response to rapid reintroduction of $[Ca^{2+}]_o$ in the presence of thapsigargin ($n = 7$) was determined as above. We found the presence of verapamil during rapid reintroduction of

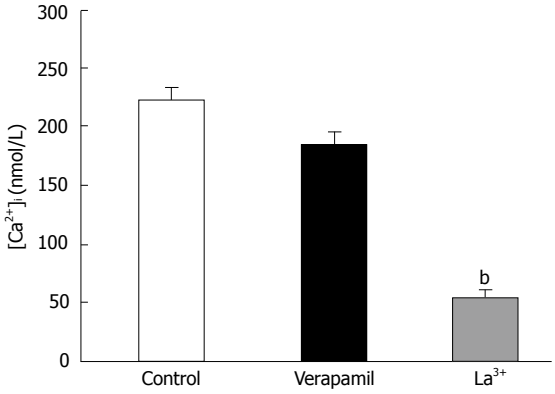

Figure 1 Ca^{2+} influx through SOCCs in enzymatically dissociated smooth muscle cells of rats distal colon. After depletion of the SR by thapsigargin in the absence of extracellular Ca^{2+}, subsequent rapid reintroduction of extracellular Ca^{2+} resulted in activation of CCE *via* SOCCs. CCE were insensitive to verapamil, but blocked by La^{3+}. [b]$P < 0.01$ *vs* control.

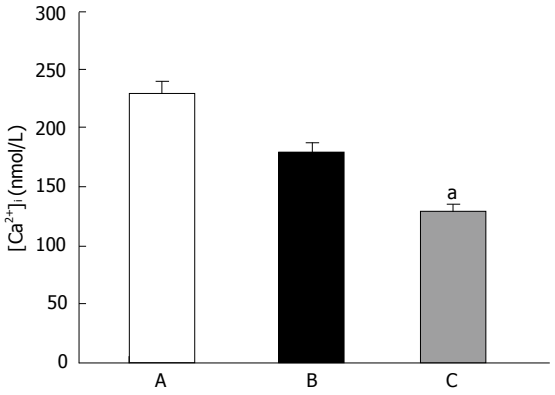

Figure 2 CCE are not mediated *via* L-type Ca^{2+} channels. (A) Controls; (B) 40 mmol/L K^+; (C) 60 mmol/L K^+. Only 60 mmol/L K^+ significantly decreased the Ca^{2+} influx mediated by CCE. [a]$P < 0.05$ *vs* control.

$[Ca^{2+}]_o$ (1.5 mmol/L) did not significantly influence Ca^{2+} influx induced by thapsigargin. Previous studies in other tissues have found that SOCC-mediated Ca^{2+} influx is inhibited by La^{3+}[20,21]. We investigated the sensitivity of multivalent cations to such Ca^{2+} influx. In a separate group of cells, pretreatment with La^{3+} and Ca^{2+} influx to rapid reintroduction in the presence of thapsigargin was greatly changed (Figure 1). Taken together, these results suggest the observed influx of extracellular Ca^{2+} in both instances was not mediated *via* L-type Ca^{2+} channels, but capacitative Ca^{2+} entry through SOCCs.

Role of membrane potential

A potential confounding factor in SOCC-mediated Ca^{2+} influx is the membrane potential, which alters the driving forces for Ca^{2+} entry. Accordingly, separate experiments were performed to determine the role of membrane potential. In the presence of verapamil (5 μmol/L), and pretreatment with high KCl solution (final concentrations of 40, 60 mmol/L), cells were re-exposed to thapsigargin, and $[Ca^{2+}]_o$ was then rapidly reintroduced (final concentration, 1.5 mmol/L). Figure 2 shows the presence of 40 mmol/L KCl did not significantly influence Ca^{2+} influx induced by

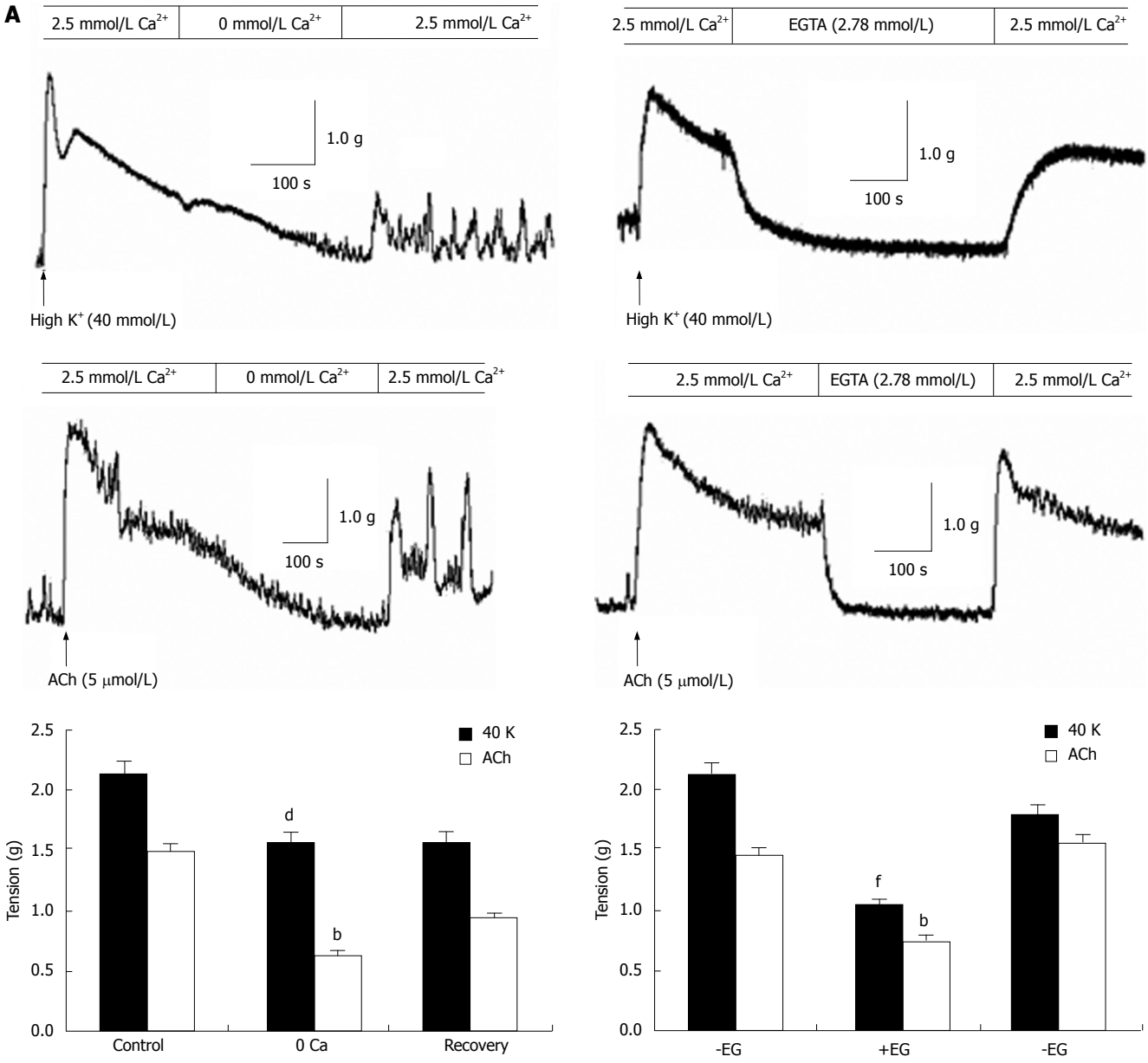

Figure 3 Role of Ca^{2+} in rat distal colon contraction. **A**: Representative results showing that removal of extracellular Ca^{2+} abolished contraction induced by high K^+ and 5 μmol/L ACh. Summarized data showing that tension induced by high K^+ and 5 μmol/L ACh before, during and after application of Ca^{2+}-free Krebs solution. $^bP < 0.01$, $^dP < 0.001$ *vs* Control; **B**: Representative results showing that chelation of extracellular Ca^{2+} with 2.78 mmol/L EGTA abolished contraction induced by high K^+ and 5 μmol/L ACh. Summarized data showing that tension induced by high K^+ and 5 μmol/L ACh in the presence of EGTA $^bP < 0.01$, $^fP < 0.001$ *vs*-EG (before).

thapsigargin. However, in the presence of 60 mmol/L KCl, Ca^{2+} influx was decreased ($P < 0.05$).

Requirement of extracellular Ca^{2+} for distal colon contraction

In isolated rat distal colon, removal of extracellular Ca^{2+} almost abolished the contraction induced by 40 mmol/L KCl or 5 μmol/L ACh, a muscarinic receptor agonist ($n = 11$, Figure 3A). Chelation of extracellular Ca^{2+} with 2.78 mmol/L EGTA, which decreases free Ca^{2+} concentration to 0.5 μmol/L in Krebs solution containing 2.5 mmol/L $CaCl_2$, had the same inhibitory effect on contraction induced by 40 mmol/L K^+ or 5 μmol/L ACh ($n = 7$, Figure 3B). Furthermore, extracellular application of the L-type VOCC blocker verapamil (5 μmol/L) almost abolished the 40 mmol/L K^+-induced tension. The tension decreased from 2.1 to 0.52 g and the inhibitory rate was 74% ($n = 7$, $P < 0.001$). The tension induced by 5 μmol/L ACh was also inhibited, but the inhibitory rate was only 41%. The tension decreased from 1.44 to 0.85 *g* ($n = 7$, $P < 0.05$) (Figure 4). These observations indicated ACh induced rat distal colon contraction by activating multiple Ca^{2+} entry pathways, whereas 40 mmol/L K^+-induced rat distal colon contraction was solely dependent on the membrane-depolarization-mediated opening of VOCCs.

CCE-mediated distal colon contraction

In the absence of extracellular Ca^{2+}, ACh induced a transient contraction that was apparently due to Ca^{2+} release from the SR. The muscarinic receptor blocker atropine (10 μmol/L) was applied to the distal colon when the ACh-induced contraction returned to baseline.

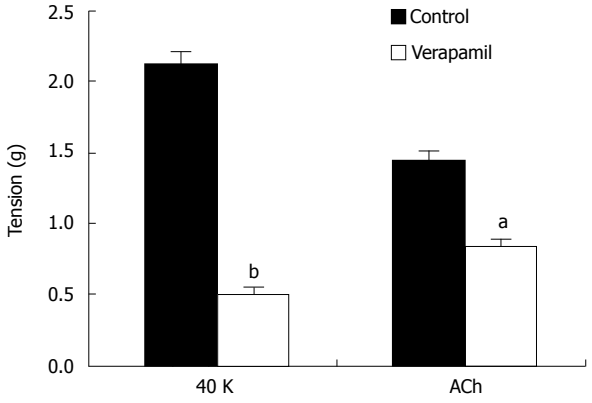

Figure 4 Effects of the VOCC blocker verapamil on distal colon contraction induced by high K^+ and ACh. $^aP < 0.05$, $^bP < 0.001$ *vs* control.

In the presence of atropine, restoration of extracellular Ca^{2+} caused tonic contraction (Figure 5Aa). We further showed tonic contraction was unaffected by verapamil ($n = 5$, Figure 5Ab). The CCE-mediated contraction in the presence of atropine was about 0.46-fold greater than that induced by SR Ca^{2+} release and the total peak contraction induced by ACh in the presence of extracellular Ca^{2+} ($n = 9$, Figure 5B). Taken together, these results showed that Ca^{2+} release from the SR and CCE *via* SOCCs both contribute to agonist-mediated distal colon contraction.

Inhibitory effects of La^{3+} on CCE-mediated distal colon contraction

La^{3+} has been shown to block SOCCs in many cell types[11,31]. La^{3+} (50 μmol/L) was applied to the distal colon when CCE-induced contraction was maximized, and the CCE-mediated contraction was significantly attenuated ($n = 7$, Figure 6A and B). These results indicated the properties of SOCCs in the distal colon are similar to those found in other tissues.

DISCUSSION

The results of this study reveal smooth muscle cells of rat distal colon express functional SOCCs, which play a vital role in agonist-induced contraction.

In many cells, depletion of intracellular Ca^{2+} stores is coupled to activation of CCE. The CCE model has gained support from experiments that used inhibitors of sarcoplasmic endoplasmic reticulum Ca^{2+}-ATPase (SERCA), such as thapsigargin[22,23]. These drugs cause passive store depletion of Ca^{2+} by blocking the reuptake of Ca^{2+} into the SR storage pools[24,25]. Studies in a variety of preparations have demonstrated that SERCA inhibitors increase Ca^{2+} influx and/or smooth muscle tone[26,27]. To investigate the role of CCE in the contractile response in rat distal colon smooth muscle, freshly dissociated cells were incubated with thapsigargin in Ca^{2+}-free HEPES/Ringer solution, followed by reintroduction of extracellular Ca^{2+}, which greatly elevated $[Ca^{2+}]_i$. The lack of effect of thapsigargin resulted in only a slightly elevated $[Ca^{2+}]_i$. Moreover, the properties of the pathway were consistent with the CCE through SOCC found in several types of non-excitable cells. They are insensitive to verapamil

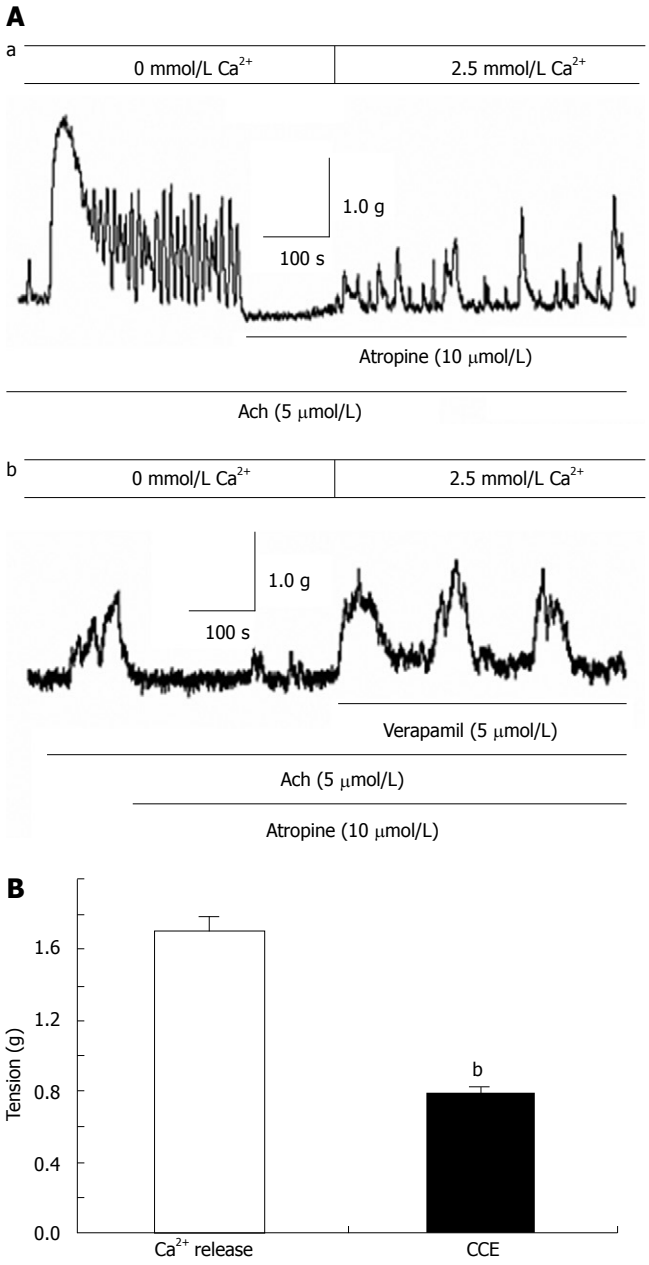

Figure 5 Contribution of Ca^{2+} release from SR stores and Ca^{2+} influx *via* CCE in ACh-induced distal colon contraction. **Aa**: In the presence of atropine, restoration of extracellular Ca^{2+} induced contraction, most likely due to Ca^{2+} influx *via* CCE; **Ab**: VOCC blocker verapamil (5 μmol/L) negligibly affected CCE-mediated contraction in the presence of atropine; **B**: Summarized data showing that CCE-mediated contraction is about 0.46-fold greater than the contraction induced by SR Ca^{2+} release in ACh-induced rat distal colon contraction. $^bP < 0.001$ *vs* Ca^{2+} release.

(L-type VOCC inhibitor), but sensitive to La^{3+} (SOCC inhibitor). Unlike VOCCs, SOCCs do not appear to be greatly affected by changes in membrane potential. If thapsigargin-induced elevation of $[Ca^{2+}]_i$ in the presence of verapamil is mediated *via* VOCCs, most positive membrane potentials should increase Ca^{2+} influx. However, in this study, such Ca^{2+} influx was not significantly altered by 40 mmol/L K^+. Nevertheless, we do not rule out the role of VOCCs in distal colon smooth muscle cells, because 60 mmol/L K^+ greatly altered elevation of $[Ca^{2+}]_i$. The relative contribution of VOCCs and SOCCs depends on whether a specific agonist causes membrane potential depolarization and/or only intracellular Ca^{2+} release.

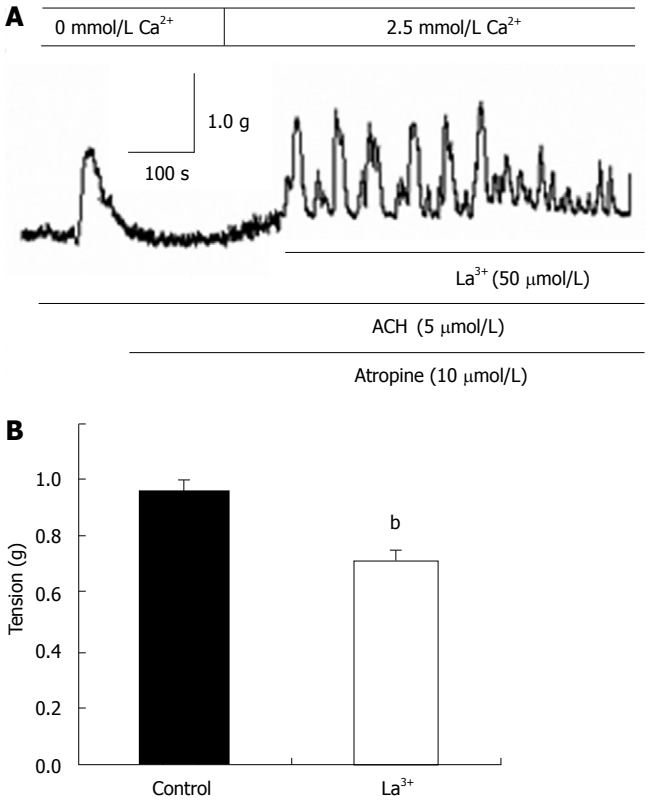

Figure 6 Inhibitory effects of La^{3+} on CCE-mediated distal colon contraction. $^bP < 0.01$ *vs* control. Control: CCE-mediated contraction.

In the rat distal colon, ACh and high K^+ induced the contraction of smooth muscle in a phasic and tonic phase. It has been suggested the phasic contraction is induced by Ca^{2+} released from the SR, whereas the tonic phase is induced by Ca^{2+} that enters from outside the cell[28]. Both pathways for inducing contraction were found in the rat distal colon in the present study. The tonic phase was abolished or significantly inhibited by removal of extracellular Ca^{2+} or chelation of extracellular Ca^{2+} with 2.78 mmol/L EGTA. The present results suggest extracellular Ca^{2+} is necessary for the contraction induced by high K^+ and ACh. ACh studied in the rat distal colon activates Ca^{2+} influx, which may be mediated by different mechanisms, from outside of the smooth muscle cells.

Having established in the present study that the contractile response to ACh in rat distal colon smooth muscle is dependent on extracellular Ca^{2+} influx, we then investigated the contributions of VOCCs and non-VOCCs to this response. Tissue pretreatment with verapamil essentially abolished the 40 mmol/L K^+-induced contraction, but only partially attenuated the response to 5 μmol/L ACh, and the inhibitory rate was 41%. These results indicate the contraction induced by high K^+ is mainly dependent on Ca^{2+} release from SR stores and Ca^{2+} influx mediated by VOCCs. Ca^{2+} influx mediated *via* VOCCs and non-VOCCs is most important for the contraction of rat distal colon smooth muscle induced by ACh.

The muscarinic receptor plays a key role in the parasympathetic nervous control of various peripheral tissues including the gastrointestinal tract[29]. Pharmacological characterization of the contractile response to ACh has suggested the $M_3/G_{q/11}/PLC\beta$ pathway, which acts through the production and release of inositol 1,4,5-trisphosphate (IP_3) to produce mobilization of stored Ca^{2+}, is largely responsible for the contraction[28]. Rather, it is believed the depletion of intracellular Ca^{2+} stores by IP_3 generates an as-yet-uncharacterized signal that activates CCE[30,31]. Because in the present study, ACh induced transient contraction that was apparently due to Ca^{2+} release from the SR in the absence of extracellular Ca^{2+}, the question arises, does CCE has any role in distal colon motility? We found that after the ACh-mediated transient contraction in the absence of extracellular Ca^{2+} and the return to baseline, the subsequent restoration of extracellular Ca^{2+} induced a sustained contraction in the presence of the muscarinic-receptor blocker atropine. Such sustained contraction was not sensitive to verapamil, and was decreased by 50 μmol/L La^{3+}. This contraction was independent of: (1) activation of the muscarinic receptor, (2) intact endothelium, and (3) opening of VOCCs. The result indicated that in the absence of extracellular Ca^{2+}, ACh actively depleted Ca^{2+} from the SR by increasing IP_3 production; therefore, activation of SOCCs and induction of CCE may be the trigger for this contraction. Taken together, the results shows that Ca^{2+} release from the SR is responsible for the phasic contraction, and that CCE *via* SOCC is necessary for the sustained contraction of ACh-induced rat distal colon contraction.

In summary, this study demonstrated the predominant source of activator Ca^{2+} for the contractile response to ACh is extracellular Ca^{2+}, and that intracellular Ca^{2+} has little role to play in mediating excitation-contraction coupling by ACh in rat distal colon smooth muscle *in vitro*. Influx of extracellular Ca^{2+} is mainly mediated by VOCCs, ROCCs and SOCCs. The study of Takeuchi *et al*[32] has shown that the source of Ca^{2+} in smooth muscle cells in the guinea pig proximal colon is mainly dependent on VOCCs. It is postulated that the source of activator Ca^{2+} for the contractile response varies among different regions of the gastrointestinal tract and the animals used. Further studies will hopefully be helpful in developing new drugs with unique properties, such as those that inhibit CCE mediated Ca^{2+} influx.

COMMENTS

Background

Various agonists induce contraction in a variety of smooth muscle preparations, with a concomitant increase in $[Ca^{2+}]_i$. It is suggested the sources of Ca^{2+} for phasic and tonic contraction induced by activation of muscarinic receptors differ among regions of the digestive tract and species of animals used.

Research frontiers

Intracellular Ca^{2+} is a critical second messenger responsible for linking external stimuli to contraction, proliferation and gene expression. It has been suggested some of the motor changes associated with intestinal infection, stress and inflammation are associated with alterations in $[Ca^{2+}]$. Further studies of pharmacological manipulation of Ca^{2+} channels may be therapeutically useful in treating colonic motility disorders.

Innovations and breakthroughs

In this study, we investigated the importance of Ca^{2+} influx in smooth muscle contraction. We also evaluated the contribution of the different Ca^{2+} influx mechanisms and the release of Ca^{2+} from the sarcoplasmic reticulum (SR) to

contraction induced by activation of muscarinic receptors in rat distal colon *in vitro*. The results showed that Ca^{2+} necessary for contractile response in the distal colon of rat was supplied from outside the smooth muscle cells via voltage-operated Ca^{2+} channels (VOCCs), receptor-operated Ca^{2+} channels (ROCCs) and store-operated Ca^{2+} channels (SOCCs), and the SR had a minor role in the contractile response.

Applications

The results provide further evidence for the contraction of the rat distal colon, which hopefully will be of importance in developing new drugs with unique properties, such as those that inhibit capacitative Ca^{2+} entry (CCE)-mediated Ca^{2+} influx.

Terminology

Thapsigargin is a potent skin irritating sesquiterpene lactone isolated from the roots of Thapsia garganica L. It also acts as a non-phorbol-ester-type tumor promoter, which discharges intracellular Ca^{2+} stores by specific inhibition of sarcoplasmic endoplasmic reticulum Ca^{2+}-ATPase (SERCA).

Peer review

This paper examined the relative contributions of internal calcium release and calcium influx to phasic and tonic contraction in the distal colon, and measured calcium concentration and calcium influx in isolated colon smooth muscle cells. The work addressed an old topic but the current discovery of SOCCs in excitable cells such as smooth muscle cell (SMC) is interesting.

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