Jpn. J. Pharmacol. 84, 431 – 437 (2000)

Role of Ca^{2+} Mobilization in Muscarinic Receptor-Mediated Membrane Depolarization in Guinea Pig Ileal Smooth Muscle Cells

Toshihiro Unno[1], Tadafumi Inaba[1], Hidenori Ohashi[1], Tadashi Takewaki[2] and Seiichi Komori[1,*]

[1]*Laboratory of Pharmacology, Department of Veterinary Medicine, Faculty of Agriculture, and* [2]*Department of Pathogenetic Veterinary Science, United Graduate School, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*

Received June 8, 2000 Accepted September 4, 2000

ABSTRACT—In single smooth muscle cells dispersed from guinea pig ileum, the muscarinic agonist carbachol (CCh) at 2 μM produced an oscillatory or sustained type of depolarization and at 100 μM, the latter type depolarization. Depletion of internal Ca^{2+} stores blocked the oscillatory response, but not the sustained responses to 2 μM and 100 μM CCh, although their decay after reaching the peak became faster. Blocking voltage-dependent Ca^{2+} channels (VDCCs) blocked both types of response to 2 μM CCh, but only slowed the initial rising phase of 100 μM CCh responses. Combination of Ca^{2+} store depletion and VDCC blockade abolished the responses to 2 μM CCh again and decreased those to 100 μM CCh in peak amplitude and persistency. Combination of Ca^{2+} store depletion with removal of extracellular Ca^{2+} markedly reduced or abolished the 100 μM CCh responses. The results suggest that muscarinic depolarization of the ileal cells requires Ca^{2+} mobilization for its generation and persistence; at weak muscarinic stimulation, both Ca^{2+} entry via VDCCs and Ca^{2+} release from internal stores may contribute to the Ca^{2+} mobilization; and under strong muscarinic stimulation, Ca^{2+} entry pathways resistant to VDCC blockers may also contribute to it.

Keywords: Intestinal smooth muscle, Muscarinic receptor, Membrane potential, Ca^{2+} mobilization, Ca^{2+} store

In intestinal smooth muscle, activation of muscarinic receptors causes depolarization of the membrane, which serves as a trigger signal for contraction (1, 2). The depolarization occurs because of the opening of nonselective cation channels (NSCCs) coupled to muscarinic receptors via a pertussis toxin-sensitive G protein (3 – 5). The opening of NSCCs is under regulation by a variety of intracellular second messengers, in which Ca^{2+} is recognized as the major regulator. This comes from voltage-clamp studies showing that carbachol (CCh)-induced current through NSCCs is strongly facilitated by a rise in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) (6 – 8). The Ca^{2+} facilitation of NSCC current is believed to play a crucial role in the muscarinic depolarization, since muscarinic receptor stimulation also increases $[Ca^{2+}]_i$.

The muscarinic increase in $[Ca^{2+}]_i$ arises through at least three different mechanisms. One is the release of Ca^{2+} from internal stores, which involves 1,4,5-trisphosphate (InsP3), a Ca^{2+}-releasing second messenger, formed by activation of phospholipase C coupled to muscarinic receptors via G proteins (5, 9). The other two are the influx of Ca^{2+} across the plasma membrane either via voltage-dependent Ca^{2+} channels (VDCCs) activated secondly by membrane depolarization due to NSCC opening or via VDCC blocker-resistant pathways such as NSCCs themselves (7) and Ca^{2+} store depletion-activated Ca^{2+} channels (7, 10).

The aim of the present study is to assess the role of these Ca^{2+}-mobilizing mechanisms in the muscarinic depolarization. To do this, we recorded changes in membrane potential from single intestinal smooth muscle cells using the nystatine-perforated patch clamp technique; and we investigated the responses to CCh under a variety of conditions where Ca^{2+} entry via VDCCs, function of Ca^{2+} stores or both were pharmacologically blocked or extracellular Ca^{2+} was removed either solely or in combination with Ca^{2+} store depletion. CCh was used at concentrations of 2 and 100 μM. Calculations of fractional receptor occupancy, based on the disociation constant for CCh at muscarinic receptors (15 μM) (11), showed that at 2 and 100 μM, the agonist is expected to occupy about 10% and 90% of the

*Corresponding author. FAX: +81-58-293-2942
E-mail: skomori@cc.gifu-u.ac.jp

receptors, respectively.

MATERIALS AND METHODS

Preparation of cells

Male guinea pigs, weighing 350 – 450 g, were stunned and killed by exanguination. A 10- to 15-cm length of the ileum was removed and divided into 3-cm segments. The longitudinal muscle layer of the segments was peeled from the underlying circular muscle and washed in physiological salt solution (PSS, composition given below). Smooth muscle cells of the muscle layers were dispersed by a combination of collagenase (0.2 – 0.6 mg/ml) and papain (0.3 – 0.6 mg/ml), as described previously (5). The cells were suspended in PSS containing 0.5 mM Ca^{2+}, and 0.1-ml aliquots were placed on glass coverslips (15 mm in diameter) and kept at 4°C until use on the same day.

Recording of membrane potential

A coverslip with single isolated ileal muscle cells was placed in a 0.5-ml organ bath on the stage of an inverted microscope, and the organ bath was washed with PSS to remove contaminants in the cell suspension and then filled with PSS in order for the cells to be equilibrated with the solution.

Cells were held under the whole-cell patch clamp with the nystatin perforated-technique, as previously described elsewhere (12). Patch pipettes with a tip resistance of 4 – 6 MΩ were filled with a KCl-based solution (composition given below) and used for recording membrane potential. Changes in membrane potential were measured via a patch-clamp amplifier (CEZ-2400; Nihon Kohden, Tokyo), stored on a PCM data recorder (RD-111T; TEAC, Tokyo) and replayed onto a thermal array recorder (RTA-1100M, Nihon Kohden) for analysis and illustration. Experiments were performed at room temparature (22°C to 25°C).

Values in the text are given as the mean ± S.E.M. Statistical significance was tested using Student's unpaired *t*-test and differences were considered significant when $P<0.05$.

Solution and drugs

The PSS used in the experiments had the following composition: 134 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 14 mM glucose and 10.5 mM HEPES (titrated to pH 7.2 with NaOH). A Ca^{2+}-free solution was prepared by substituting the $CaCl_2$ (2 mM) with equimolar $MgCl_2$ and adding 0.5 mM EGTA to it. The KCl-based pipette solution had the following composition (mM): 134 mM KCl and 10.5 mM HEPES (titrated to pH 7.2 with KOH), to which nystatin dissolved in DMSO (40 mg/ml) was added to give a final concentration of 0.2 mg/ml.

Drugs used were thapsigargin, nystatin and nicardipine (Sigma, St. Louis, MO, USA); caffeine and *O,O'-bis* (2-aminoethyl)ethyleneglycol-*N,N,N'-N'*-tetraacetic acid (EGTA) (Wako, Osaka); CCh and nifedipine (Tokyo Kasei, Tokyo).

Drugs were applied by replacing the bath solution with drug-containing PSS several times within 10 s. Exchange of the bath solution with Ca^{2+}-free PSS was also performed in a similar way.

RESULTS

CCh-induced membrane depolarization

Single ileal cells had a resting membrane potential of -52.8 ± 1.5 mV (n = 60) with cell-to-cell variations from -35 up to -65 mV. A small fraction (8.3%) of the cells exhibited spontaneous electrical activity that generated sporadic discharges of spike potentials with or without overshoot (see Fig. 1A).

CCh (2 μM) produced oscillatory changes in membrane potential in 5 of the 13 cells tested (Fig. 1A) and a sustained depolarization with no oscillatory component in the 8 remaining cells (Fig. 1B). In the oscillatory type of response, membrane potential oscillations were superimposed on a sustained depolarization whose size varied within the range from a few mV up to 20 mV during their generation. The individual oscillations were 10 to 40 mV in size and 2 to 4 s in duration, and they often carried spike potentials with or without overshoot on their rising phase (see the inset in Fig. 1A). The interoscillation interval was more or less constant, being between 7 and 10 s. In the sustained type of response, the depolarization reached a peak in 10 to 30 s after its onset, and then maintained the peak level or gradually declined until the agonist was washed away 80 – 120 s later. The size of depolarizations measured as the difference between the peak level and the level before CCh application (hereafter referred to as the peak size) varied from about 25 to 45 mV in different cells, giving a mean value of 34.3 ± 8.2 mV (n = 8). Both the oscillatory and sustained type of responses were usually initiated by discharge of one or a few spikes (Fig. 1: A and B).

As shown in Fig. 1C, 100 μM CCh produced a membrane depolarization similar in shape to the sustained type of response to 2 μM CCh in all 6 cells tested. However, the depolarization rose more rapidly, because its rising phase was formed by the upstroke of a single spike. The peak size of depolarizations was 43.4 ± 4.3 mV (n = 6) on average, which was greater (but not significantly) than the corresponding value for 2 μM CCh (34.3 ± 8.2 mV). The effect of CCh was blocked by pretreatment with 1 μM atropine, indicating its mediation by muscarinic receptors.

Unlike spike potentials, the oscillatory and sustained components of CCh-evoked depolarizations never exceeded the zero potential level but approached it, in agreement with the reversal potential for CCh-evoked NSCC current,

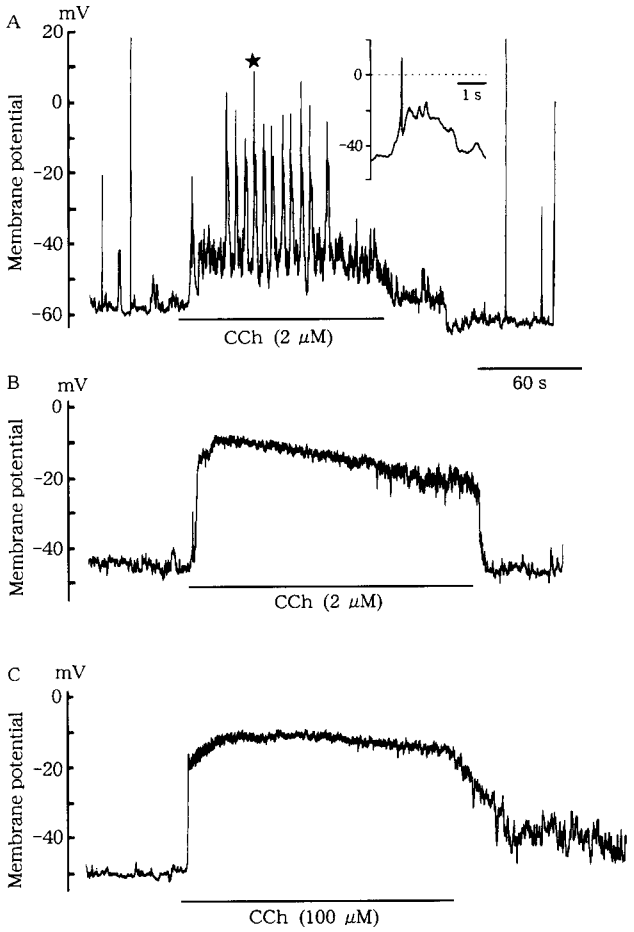

Fig. 1. Changes in membrane potential of single ileal smooth muscle cells in response to carbachol (CCh). Cells were bathed in normal PSS and held under the current clamp mode using the nystatin-perforated patch clamp technique. CCh was applied as indicated by the solid lines below recording traces. A and B: recording traces from two different cells exposed to 2 μM CCh; one of which responded with an oscillatory type of depolarization (A) and the other with a sustained type of it (B). The inset in panel A shows a time-expanded trace of an oscillation marked ★; note that the oscillation carried an overshoot spike on its rising phase. C: recording traces from another cell exposed to 100 μM CCh, which responded with a sustained depolarization.

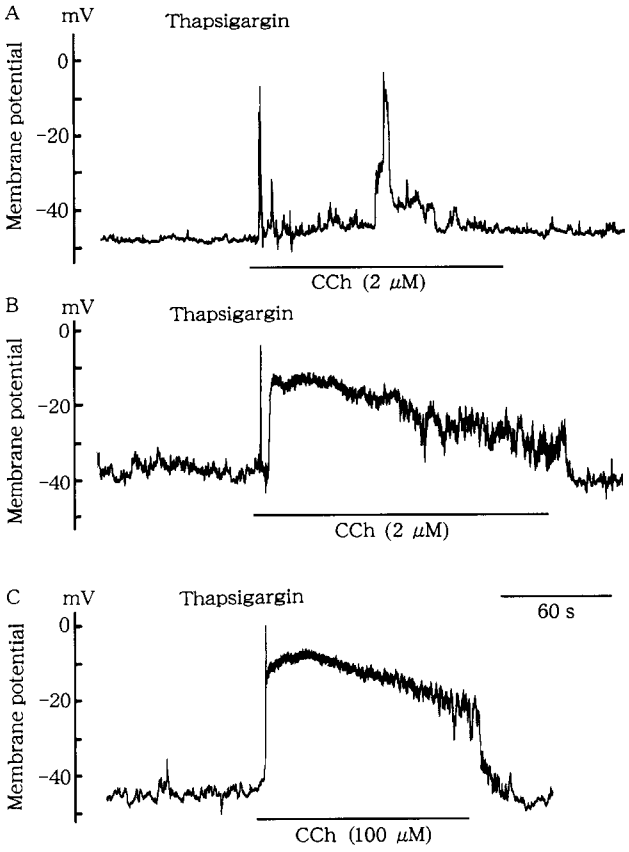

Fig. 2. Effect of thapsigargin on the depolarizing effect of CCh. Thapsigargin (1 μM) was added in the bath solution 3 min before application of CCh. A, B and C: recording traces from thapsigargin-treated cells exposed to 2 μM CCh (A and B) and 100 μM CCh (C). Note that CCh did not produce the oscillatory type of depolarization and that in B and C, the CCh-evoked sustained depolarizations declined more rapidly after reaching their peak than in control cells (see Fig. 1: B and C).

which was -5 to -10 mV under such ionic environments as used here (3).

Application of CCh was not repeated in one cell, because of deterioration of cells and/or desensitization of CCh responses, and the effects of 2 and 100 μM CCh described above were used as controls in the following experiments.

Efffect of depletion of internal Ca^{2+} stores

Cells were treated with 1 μM thapsigargin, a specific inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPases, for 3 min before and throughout application of CCh. In preliminary experiments, such treatment with thapsigargin was found to cause depletion of internal stores, because caffeine (10 mM), which releases Ca^{2+} from internal stores (5, 9), was made ineffective in inducing Ca^{2+}-activated K^+ current (n = 3). Thapsigargin treatment did not significantly change the resting membrane potential (-47.9 ± 3.4 mV, n = 11).

In tapsigargin-treated cells (n = 8), 2 μM CCh did not produce the oscillatory type of depolarization, but ensured a sustained depolarization. In 4 of the 8 cells, the sustained depolarization was as small as <10 mV and sometimes less stable because of discharges of a burst of spikes that summed into a brief, large depolarization (Fig. 2A). In the 4 remainig cells, it resembled the sustained type of response in the control cells, as shown in Fig. 2B, although its peak size (28.5 ± 5.5 mV, n = 4) was smaller (but not significantly) than the control value (34.3 ± 8.2 mV, n = 8). It also tended to decrease more rapidly after reaching its peak. When the size of depolarizations after the elapse of 60 s

from the peak (the 60 s-later size) was expressed as percentage of the peak size, it was estimated to be 43.2 ± 7.3% (n = 4). This mean value was significantly smaller ($P<0.05$) than the corresponding value for the control responses (79.5 ± 3.6%, n = 8).

When 100 μM CCh was used, as shown in Fig. 2C, it produced a sustained depolarization with a peak size of 42.7 ± 3.0 mV (n = 7), which was very similar to the control value (43.4 ± 4.3 mV, n = 6). However, the 60 s-later size (55.9 ± 10.4%, n = 7) was significanly smaller ($P<0.05$) than the control (84.2 ± 5.1%, n = 6).

Effect of blocking voltage-dependent Ca^{2+} channels (VDCCs)

To block Ca^{2+} influx via VDCCs, cells were treated with 1 μM nicardipine for 1 min before and throughout CCh application. Preliminary experiments showed that such treatment with nicardipine completely blocked Ca^{2+} current evoked by step depolarizations (n = 4).

In nicardipine-treated cells, 2 μM CCh produced no appreciable depolarization (n = 6) (Fig. 3A), whereas 100 μM CCh produced a sustained depolarization with a peak size of 39.6 ± 3.8 mV and a 60-s later size of 78.1 ± 5.0% (n = 7) (Fig. 3B). Neither of the mean values significantly differed from the corresponding control values (43.4 ± 4.3 mV and 84.2 ± 5.1%, n = 6). A noticeable change was the abolition of spike potentials that overlapped the initial rising phase of the depolarizations, so that the maximum rate of rise was significantly decreased to 93.7 ± 12.8 mV/s (n = 7) from 233.2 ± 37.7 mV/s (n = 6) after nicardipine treatment ($P<0.05$). Simlar results were obtained with nifedipine (1 μM), used instead of nicardipine (n = 2).

Effect of combination of Ca^{2+} store depletion and VDCC blockade

To achieve both depletion of Ca^{2+} stores and blockade of VDCCs at the same time, cells were treated with thapsigargin and nicardipine in similar ways as those mentioned above. The combined treatment caused no significant change in the resting potential (−51 ± 3.8 mV, n = 9).

In such cells, 2 μM CCh again produced no appreciable depolarization (data not shown, n = 3), whereas 100 μM CCh produced a sustained depolarization (Fig. 3C). Mean values for the peak size and 60 s-later size were 26.3 ± 5.8 mV and 40.5 ± 9.3% (n = 6), respectively, each of which was significantly smaller ($P<0.05$) than the corresponding control value (see above).

Effect of removing extracellular Ca^{2+} and its combination with Ca^{2+} store depletion

The depolarizing effect of 100 μM CCh was only partially inhibited by the combination of Ca^{2+}-store depletion and VDCC blockade, so it was further investigated under different conditions.

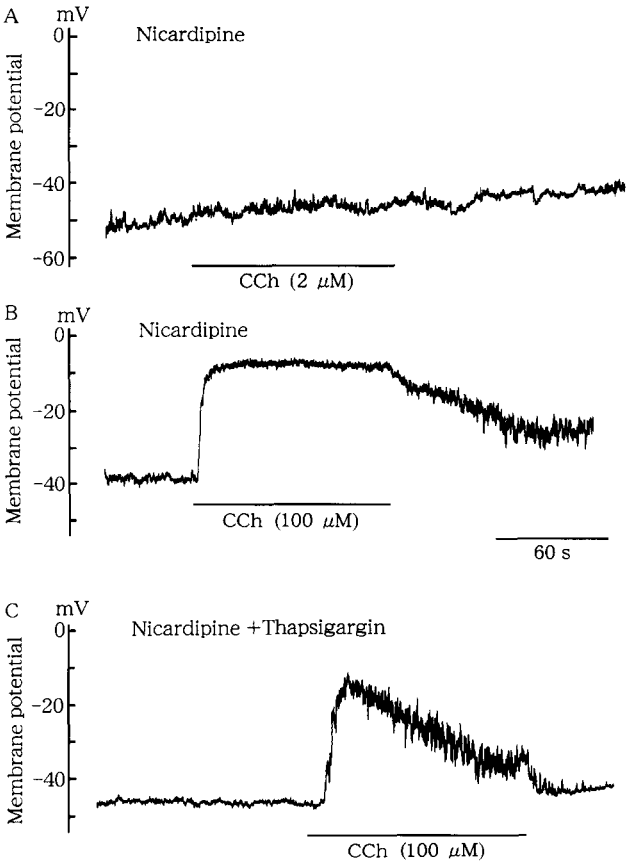

Fig. 3. Effects of nicardipine and its combination with thapsigargin on the depolarizing effect of CCh. Membrane potential changes produced by CCh in cells treated with nicardipine. Nicardipine (1 μM) was added in the bath solution 1 min before application of CCh, and thapsigargin (1 μM) was added in the same way as described in Fig. 2. A and B: recording traces from nicardipine-treated cells exposed to 2 and 100 μM CCh, respectively. C: recording trace from a nicardipine and thapsigargin-treated cell exposed to 100 μM CCh. Note abolition by nicardipine of the response to 2 μM CCh. See the text for details.

To eliminate Ca^{2+} influx via all possible pathways including VDCCs, the bath solution was changed from normal PSS to a Ca^{2+}-free PSS at 30 to 40 s before application of 100 μM CCh. The removal of extracellular Ca^{2+} hardly had any effect on the resting membrane potential (−53.1 ± 4.1 mV, n = 10). Under such conditions, the CCh produced a sustained depolarization in all 6 cells tested (Fig. 4A). Mean values for the peak size and 60 s-later size were 29.4 ± 4.5 mV and 54.7 ± 9.0% (n = 6), respectively, either of which was significantly smaller ($P<0.05$) than the corresponding control value.

In addition to their immersion in the Ca^{2+}-free PSS, cells were treated with 1 μM thapsigargin to deplete internal Ca^{2+} stores. Such combined procedures finally prevented 100 μM CCh from producing any appreciable depolarization (n = 5), as shown in Fig. 4B.

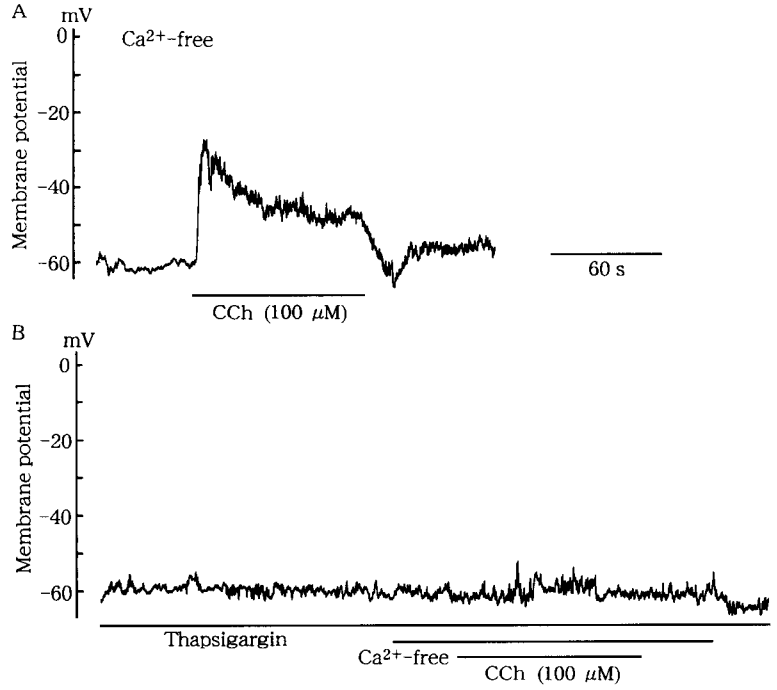

Fig. 4. Effects of removing extracellular Ca^{2+} and of its combination with thapsigargin treatment on the depolarizing effect of 100 μM CCh. The extracellular Ca^{2+} was removed 30 s before application of CCh by changing the bath solution from normal PSS to a Ca^{2+}-free PSS containing 0.5 mM EGTA. Thapsigargin was added in the bath solution as described in Fig. 2. A and B: recording traces from cells immersed in the Ca^{2+}-free solution (A) and additionally treated with thapsigargin (B). Note that combination of removal of extracellular Ca^{2+} and thapsigargin treatment prevented 100 μM CCh from producing any appreciable change in membrane potential.

DISCUSSION

In single ileal smooth muscle cells, we observed the effects on membrane potential of 2 and 100 μM CCh, which were expected to occupy about 10 and 90% of muscarinic receptors, respectively (see Introduction). The former CCh produced the oscillatory or sustained type of depolarization, and the latter CCh usually produced the sustained type of depolarization. There was no significant difference in the peak size between the sustained responses at the two concentrations. In addition, individual oscillations of the oscillatory response occasionally had a peak size similar to the sustained response. These observations imply that muscarinic stimulation as weak as only 10% receptor occupancy by CCh can cause full-size depolarization. However, it was found that the responses to weak and strong muscarinic stimulations differed in the sensitivity to various procedures that affect Ca^{2+} mobilization.

The depolarizing effect of 2 μM CCh was blocked by the VDCC blocker nicardipine, suggesting that Ca^{2+} entry into the cell via VDCCs is essential for membrane depolarization arising from weak muscarinic stimulation. Since the opening of muscarinic receptor-operated NSCCs is very sensitive to a rise in $[Ca^{2+}]_i$ (6, 7), it is highly probable that during weak muscarinic stimulation, a small depolarization is initially produced by a small degree of NSCC opening to generate action potentials, whereby the Ca^{2+} entering the cell via VDCCs further facilitates NCCC opening directly or indirectly through triggering a Ca^{2+} release from internal stores (see below) and produces a greater depolarization. Such a positive feedback mechanism may explain why contraction evoked by submicromolar concentrations of CCh is virtually abolished by VDCC blockers (13).

Nicardipine or its analogue, nifedipine, only slowed the initial rising phase of 100 μM CCh-evoked depolarization. Thus, the importance of VDCC-dependent Ca^{2+} mobilization in the response to strong muscarinic stimulation may be restricted to its early phase. This view is supported by our previous observation that Ca^{2+} current evoked by step depolarizations was markedly inhibited within 10 s or so after the beginning of CCh application at 10 μM or more (14).

Depletion of internal Ca^{2+} stores with thapsigargin selectively blocked the CCh effect in producing the oscillatory depolarization, as previously reported by Kohda et al. (15). As CCh causes $[Ca^{2+}]_i$ oscillation through InsP3-induced cyclical release of Ca^{2+} from internal stores (9), this type of Ca^{2+} mobilization seems likely to be essential for

generation of the oscillatory depolarization, which is supported by the result from voltage clamp studies that 1 to 2 μM CCh often produces oscillations of NSCC current (5, 8, 16). The question as to why the VDCC blocker abolished the oscillatory depolarization would be explained on the assumption that the Ca^{2+} incoming via VDCCs can potentiate a subthreshold action of InsP3 formed by weak muscarinic stimulation, leading to a massive release of Ca^{2+} from the store (15). Another major effect of the Ca^{2+} store depletion was to decrease the sustained response to 2 and 100 μM CCh in the 60-s later size but not the peak size, suggesting that functional Ca^{2+} stores play a crucial role in maintaining the muscarinic depolarization. It is postulated that during muscarinic receptor activation, Ca^{2+} stores are undergoing restless uptake and release of Ca^{2+} to keep $[Ca^{2+}]_i$ at an increased level. Inhibition of Ca^{2+}-store pumps by thapsigargin may disturb the Ca^{2+} uptake-release process, resulting in reduction of the Ca^{2+} supply for facilitation of NSCC activity.

Our previous voltage clamp studies showed that application of 100 μM CCh immediately causes a rapid, massive release of stored Ca^{2+} to facillitate NSCC opening (8, 16). However, in the present study, no significant decrease in the peak size of 100 μM CCh-evoked depolarizations occurred after Ca^{2+}-store depletion. This can be explained by assuming that the remaining mechanisms for Ca^{2+} mobilization, such as Ca^{2+} entry via VDCCs and other pathways, can still provide a sufficient amount of Ca^{2+} for facillitation of NSCC opening, resulting in a full-size depolarization. An analogous explanation is also applicable to the failure of VDCC blockade to decrease the peak size of the CCh-evoked depolarizations.

In the absence of both Ca^{2+} entry via VDCCs and Ca^{2+} store release, 100 μM CCh was still effective in producing a sustained depolarization, but the effect was completely blocked when Ca^{2+} entry via all other possible pathways was additionally eliminated (see Fig. 4B). Therefore, in the case of strong muscarinic stimulation, it is likely that VDCC blocker-resistant Ca^{2+} entry pathways may also operate to contribute to membrane depolarization. Muscarinic receptor-operated NSCCs themselves might be responsible for the Ca^{2+} entry pathway, although Ca^{2+} enrty through the NSCCs is too small to increase $[Ca^{2+}]_i$ under physiological conditions (1.5 – 2 mM external Ca^{2+}) (7). If the Ca^{2+} entry via NSCCs causes a localized, significant increase in the subsarcolemmal Ca^{2+} concentration, NSCC opening can be facilitated in a positive-feedback manner. Another likely Ca^{2+} entry pathway is one activated by Ca^{2+} store depletion (7, 10). Further study is needed to determine whether Ca^{2+} influx via these pathways is significant for the muscarinic depolarizing response.

Bolton (1) recorded changes in membrane potential in ileal longitudinal muscle layer using the intracellular microelectrode technique and showed that a near-maximum effect of CCh in producing sustained depolarizations was attained at 1.4 μM, a result similar to the present result obtained from single ileal cells. He also found that CCh at submicromolar concentrations caused membrane potential to oscillate through cyclical increase in membrane cationic conductance (17). The effect of CCh could be explained based on the present result that CCh-induced oscillation of membrane potential involves both VDCC- and InsP3-dependent Ca^{2+} mobilizations (also see refs. 8 and 15). Recently, Cousins et al. (18) found in the ileal longitudinal muscle that under blockade of VDCCs, brief transmural stimuli initiate a cholinergic excitatory junction potential with a rise in $[Ca^{2+}]_i$. Based on the present result that strong, but not weak, muscarinic stimulation produces VDCC blocker-resistant depolarizations, their finding implies that a population of smooth muscle cells may be exposed to relatively high concentrations of acetylcholine upon generation of nerve impulses.

In gastrointestinal smooth muscle, it is well documented that the muscarinic contraction underlies both membrane depolarization due to NSCC activation and InsP3-induced release of stored Ca^{2+}, and the NSCC activation and Ca^{2+} release have been suggested to be mediated by M2 and M3 receptors, respectively (19 – 21). However, the contractile response is generally believed to be mediated by the M3 receptor (22 – 24). The present study has demonstrated that dysfunction of internal Ca^{2+} stores affects the depolarizing responses to CCh. This can be taken as evidence for the existence of cross-talks between the M2- and M3-signaling systems. To explain the above enigma, further study will be needed to understand the mechanism of the cross-talk.

In summary, the depolarizing response to muscarinic receptor stimulation requires Ca^{2+} mobilization for its initiation and persistence in guinea pig ileal smooth muscle; at weak stimulation, both Ca^{2+} entry via VDCCs and Ca^{2+} release from internal stores may take place in the Ca^{2+} mobilization with predominance of the former, and under strong stimulation, Ca^{2+} entry via VDCC blocker-resistant pathways may also take place in it, and the contribution of Ca^{2+} entry via VDCCs may be restricted to an early period of the depolarizing response.

REFERENCES

1 Bolton TB: The depolarizing action of acetylcholine or carbachol in intestinal smooth muscle. J Physiol (Lond) **220**, 647 – 671 (1972)

2 Bolton TB: Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev **59**, 607 – 718 (1979)

3 Benham CD, Bolton TB and Lang RJ: Acetylcholine activates an inward current in single mammalian smooth muscle cells. Nature **316**, 345 – 346 (1985)

4 Inoue R and Isenberg G: Acetylcholine activates nonselective cation channels in guinea-pig ileum through a G protein. Am J Physiol **258**, C1173 - C1178 (1990)
5 Komori S, Kawai M, Takewaki T and Ohashi H: GTP-binding protein involvement in membrane currents evoked by carbachol and histamine in guinea-pig ileal muscle. J Physiol (Lond) **450**, 105 - 126 (1992)
6 Inoue R and Isenberg G: Intracellular calcium ions modulate acetylcholine-induced inward current in guinea-pig ileum. J Physiol (Lond) **424**, 73 - 92 (1990)
7 Pacaud P and Bolton TB: Relation between muscarinic receptor cationic current and internal calcium in guinea-pig jejunal smooth muscle cells. J Physiol (Lond) **441**, 477 - 499 (1991)
8 Komori S, Kawai M, Pacaud P, Ohashi H and Bolton TB: Oscillations of receptor-operated cationic current and internal calcium in single guinea pig ileal smooth muscle cells. Pflugers Arch **424**, 431 - 438 (1993)
9 Komori S and Bolton TB: Calcium release induced by inositol 1,4,5-trisphosphate in single rabbit intestinal smooth muscle cells. J Physiol (Lond) **433**, 495 - 517 (1991)
10 Ohta T, Kawai K, Ito S and Nakazato Y: Ca^{2+} entry activated by emptying of intracellular Ca^{2+} stores in ileal smooth muscle of the rat. Br J Pharmacol **114**, 1165 - 1170 (1995)
11 Furchgott RF: The use of β-haloalkylamines, in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. *In* Advances in Drug Research, Vol **3**, Edited by Harer NJ and Simmons AB, pp 21 - 55, Academic Press, London (1966)
12 Kohda M, Komori S, Unno T and Ohashi H: Carbachol-induced $[Ca^{2+}]_i$ oscillations in single smooth muscle cells of guinea-pig ileum. J Physiol (Lond) **492**, 315 - 328 (1996)
13 Brading AF and Sneddon P: Evidence for multiple sources of calcium for activation of the contractile mechanism of guinea-pig taenia coli on stimulation with carbachol. Br J Pharmacol **70**, 229 - 240 (1980)
14 Unno T, Komori S and Ohashi H: Inhibitory effect of muscarinic receptor activation on Ca^{2+} channel current in smooth muscle cells of guinea-pig ileum. J Physiol (Lond) **484**, 567 - 581 (1995)
15 Kohda M, Komori S, Unno T and Ohashi H: Carbachol-induced oscillations in membrane potential and $[Ca^{2+}]_i$ in guinea-pig ileal smooth muscle cells. J Physiol (Lond) **511**, 559 - 571 (1998)
16 Zholos AV, Komori S, Ohashi H and Bolton TB: Ca^{2+} inhibition of inositol trisphosphate-induced Ca^{2+} release in single smooth muscle cells of guinea-pig small intestine. J Physiol (Lond) **481**, 97 - 109 (1994)
17 Bolton TB: On the nature of the oscillations of the membrane potential (slow waves) produced by acetylcholine or carbachol in intestinal smooth muscle. J Physiol (Lond) **216**, 403 - 418 (1971)
18 Cousins HM, Edwards FR, Hirst GDS and Wendt IR: Cholinergic neuromuscular transmission in the longitudinal muscle of the guinea-pig ileum. J Physiol (Lond) **471**, 61 - 86 (1993)
19 Zholos AV and Bolton TB: Muscarinic receptor subtypes controlling the cationic current in guinea-pig ileal smooth muscle. Br J Pharmacol **122**, 885 - 893 (1997)
20 Komori S, Unno T, Nakayama T and Ohashi H: M2 and M3 muscarinic receptors couple, respectively, with activation of nonselective cationic channels and potassium channels in intestinal smooth muscle cells. Jpn J Pharmacol **76**, 213 - 218 (1998)
21 Rhee JC, Rhee PL, Park MK, So I, Uhm DY, Kim KW and Kang TM: Muscarinic receptors controlling the carbachol-activated nonselective cationic current in guinea pig gastric smooth muscle cells. Jpn J Pharmacol **82**, 331 - 337 (2000)
22 Eglen RM, Adham N and Whiting RL: Acute desensitization of muscarinic receptors in the isolated guinea-pig ileal longitudinal muscle. J Auton Pharmacol **12**, 137 - 148 (1992)
23 Eglen RM and Harris GC: Selective inactivation of M2 and M3 receptors in guinea-pig ileum and artria in vitro. Br J Pharmacol **109**, 946 - 952 (1993)
24 Honda K, Takano Y and Kamiya H: Pharmacological profiles of muscarinic receptors in the longitudinal smooth muscle. Jpn J Pharmacol **62**, 43 - 47 (1993)