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Substance P induces inward current and regulates pacemaker currents through tachykinin NK_1 receptor in cultured interstitial cells of Cajal of murine small intestine

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Abstract

We investigated whether substance P modulates pacemaker currents generated in cultured interstitial cells of Cajal of murine small intestine using whole cell patch-clamp techniques at 30 °C. Interstitial cells of Cajal generated spontaneous inward currents (pacemaker currents) at a holding potential of −70 mV. Tetrodotoxin, nifedipine, tetraethylammonium, 4-aminopyridine, or glibenclamide did not change the frequency and amplitude of pacemaker currents. However, divalent cations (Ni^{2+}, Mn^{2+}, Cd^{2+}, and Co^{2+}), nonselective cationic channel blockers (gadolinium and flufenamic acid), and a reduction of external Na^+ from normal to 1 mM inhibited pacemaker currents indicating that nonselective cation channels are involved in their generation. Substance P depolarized the membrane potential in current clamp mode and produced tonic inward pacemaker currents with reduced frequency and amplitude in voltage clamp mode. [D-Arg^1, D-$Trp^{7,9}$, Leu^{11}] substance P, a tachykinin NK_1 receptor antagonist, blocked these substance P-induced responses. Furthermore, [Sar^9, $Met(O_2)^{11}$] substance P, a specific tachykinin NK_1 receptor agonist, depolarized the membrane and tonic inward currents mimicked those of substance P. Substance P continued to produce tonic inward currents in external Ca^{2+}-free solution or in the presence of chelerythrine, a protein kinase C inhibitor. However, substance P-induced tonic inward currents were blocked by thapsigargin, a Ca^{2+}-ATPase inhibitor in the endoplasmic reticulum or by an external 1 mM Na^+ solution. Our results demonstrate that substance P may modulate intestinal motility by acting on the interstitial cells of Cajal by activating nonselective cation channels via the release of intracellular Ca^{2+} induced by tachykinin NK_1 receptor stimulation.

Keywords: Interstitial cell of Cajal; Pacemaker current; Neurokinin receptor1; Substance P; Small intestine

1. Introduction

Gastrointestinal smooth muscles show spontaneous mechanical contractions, which are initiated by periodic membrane depolarizations, called slow waves. Slow waves determine the frequency and the timing of smooth muscle contraction (Szurszewski, 1987). In addition, slow waves control peristaltic activity by causing propagating waves of intraluminal pressure and the pulsatile flow of intestinal contents (Aros and Camilleri, 2001). The interstitial cells of Cajal are pacemaker cells that generate slow waves by producing spontaneous inward currents (pacemaker currents) (Huizinga et al., 1995; Koh et al., 1998; Ward et al., 1994). These cells are connected to each other, to form a network, and form gap junctions with smooth muscles. Therefore, the pacemaker currents induced by interstitial cells of Cajal are directly transmitted to smooth muscles through gap junctions (Sanders, 1996). In addition, the interstitial cells of Cajal are closely associated with varicos-

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ities of the enteric nerves, which mediate inhibitory and excitatory nerve signals to smooth muscles (Publicover et al., 1993; Sanders, 1996; Ward et al., 2000a,b). Therefore, these cells play an important role as basic regulators of gastrointestinal motility. Moreover, disruptions to these cells, such as, a reduction in their numbers or changes to their morphological patterns, produce pathological motility disorders in the gastrointestinal tract (Jain et al., 2003; Sanders et al., 1999; Vanderwinden and Rumessen, 1999).

Hormones and the enteric nervous system regulate gastrointestinal mechanical contractions and electrical activity by modulating these slow waves (Olsson and Holmgren, 2001). These relations suggest that the interstitial cells of Cajal are physiological and therapeutic targets for numerous hormones, neurotransmitters, and drugs. In the enteric nervous system, numerous neurotransmitters are released and regulate gastrointestinal motility. Neurotransmission is mediated not only by the classical neurotransmitters but also by neuropeptide transmitters. It is well known that the major excitatory neurotransmitters are acetylcholine and substance P, whereas the major inhibitory neurotransmitters are nitric oxide and vasoactive intestinal polypeptide (Daniel, 2001; Sanders, 1998). Substance P, neurokinin A and neurokinin B are members of the family of mammalian tachykinin peptides that are predominantly released by enteric neurons, and which exert a potent contractile effect on gastrointestinal smooth muscle through tachykinin receptors by modulating ionic channels and by producing second messengers (Bartho and Holzer, 1985; Mayer et al., 1990; Watson and Downes, 1983). Tachykinin receptors are distributed in enteric nerves and smooth muscles. In addition, a tachykinin receptor was identified in the interstitial cells of Cajal immunohistochemically (Lavin et al., 1998; Vannucchi et al., 1997), suggesting that substance P may modulate gastrointestinal motility by affecting the interstitial cells of Cajal. However, no functional study of tachykinin on the interstitial cells of Cajal has been performed. Hence, here we performed a series of functional experiments to characterize the modulation of pacemaker currents by substance P and its receptor in cultured interstitial cells of Cajal of murine small intestine.

2. Methods

2.1. Preparation of cells

Use and treatment of animals were approved by the institutional animal use and care committee at Seoul National University College of Medicine.

Balb/C mice (8–13 days old) of either sex were anesthetized with ether and sacrificed by cervical dislocation. The small intestines from 1 cm below the pyloric ring to the cecum were removed and opened along the mesenteric border. Luminal contents were washed away with Krebs–Ringer bicarbonate solution, tissues were pinned to the base of Sylgard dish, and the mucosa was removed by sharp dissection. Small strips of intestinal muscle were equilibrated in Ca^{2+}-free Hank's solution containing (in mM) KCl, 5.36; NaCl, 125; NaOH, 0.336; Na_2HCO_3, 0.44; glucose, 10; sucrose, 2.9 and HEPES, 11; and adjusted to pH 7.4 with tris for 30 min. Cells were dispersed by incubating for 15 min at 37 °C in an enzyme solution containing collagenase (Worthington Biochemical, Lakewood, NJ, USA), 1.3 mg/ml, bovine serum albumin (Sigma, St. Louis, MO, USA), 2 mg/ml, trypsin inhibitor (Sigma), 2 mg/ml and ATP, 0.27 mg/ml. The cells were then plated onto sterile glass coverslips coated with murine collagen (2.5 μg/ml, Falcon/BD) in 35 mm culture dishes, and cultured at 37 °C in a 95% O_2–5% CO_2 incubator in SMGM (smooth muscle growth medium, Clonetics, San Diego, CA, USA) supplemented with 2% antibiotics/antimycotics (Gibco, Grand Island, NY, USA) and murine stem cell factor (SCF, 5 ng/ml, Sigma). Interstitial cells of Cajal (ICCs) were identified immunologically using a monoclonal antibody for Kit protein (ACK_2) labeled with Alexa Fluor 488 (Molecular Probes, Eugene, OR, USA).

2.2. Patch-clamp experiments

The whole-cell configuration of the patch-clamp technique was used to record membrane currents (voltage clamp) and membrane potentials (current clamp) from cultured ICCs. Currents or potentials were amplified using an Axopatch 1-D (Axon Instruments, Foster, CA, USA). Command pulse was applied using an IBM-compatible personal computer and pClamp software (version 6.1; Axon Instruments). The data were filtered at 5 kHz and displayed on an oscilloscope, a computer monitor, and a pen recorder (Gould 2200, Gould, Vally view, OF, USA).

Results were analyzed using pClamp and Graph Pad Prism (version 2.01) software. All experiments were performed at 30 °C.

2.3. Solutions and drugs

The cells were bathed in a solution containing (mM): KCl, 5; NaCl, 135; $CaCl_2$, 2; glucose, 10; $MgCl_2$, 1.2 and HEPES, 10 adjusted to pH 7.2 with tris. The pipette solution contained (mM): K-aspartate, 120; KCl, 20; $MgCl_2$, 5; K_2ATP, 2.7; Na_2GTP, 0.1; creatine phosphate disodium, 2.5; HEPES, 5; EGTA, 0.1 adjusted to pH 7.2 with tris. In this condition, the estimated Cl^- equilibrium potential was −70 mV. Therefore, we recorded pacemaker currents at a holding potential of −70 mV to rule out the effect of Cl^- currents. The reducing external Na^+ was replaced by *N*-methyl-D-glucamine (NMDG).

The drugs used were: Tetrodotoxin (TTX), nifedipine, tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), glibenclamide, nickel chloride (Ni^{2+}), Manganese chloride (Mn^{2+}), cadmium chloride (Cd^{2+}), cobalt chloride (Co^{2+}), gadolinium chloride (Gd^{3+}), flufenamic acid, sub-

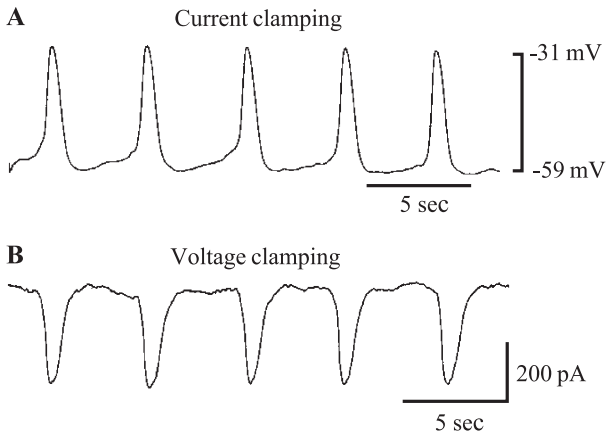

Fig. 1. Typical trace of slow waves in current clamping mode (A) and spontaneous pacemaker currents in voltage clamping mode recorded at a holding potential of − 70 mV (B) in cultured interstitial cells of Cajal from murine small intestine.

stance P ([D-Pro^2, D-Trp^{7,9}] substance P), tachykinin NK_1 receptor antagonist ([D-Arg^1, D-Trp^{7,9}, Leu^{11}] substance P), tachykinin NK_1 receptor agonist ([Sar^9, Met$(O_2)^{11}$]substance P), thapsigargin, and chelerythrine. All drugs were purchased from the Sigma.

2.4. Statistical analysis

Data are presented as means ± standard errors. Differences were evaluated using the Student's *t*-test. A *p* value of ≤0.05 was taken as a statistically significant. The *n* values reported in the text refer to the number of cells used in the patch-clamp experiments.

3. Results

3.1. Spontaneous inward pacemaker currents in the interstitial cells of Cajal

Under a current clamp, interstitial cells of Cajal generated slow waves. The resting membrane potential was − 59 ± 5 mV and the amplitude was 28 ± 2 mV (*n* = 13; Fig. 1A). Under a voltage clamp at a holding potential of − 70 mV, interstitial cells of Cajal generated spontaneous inward currents, called 'pacemaker currents'. The frequency of the pacemaker current generated was 16 ± 2 cycles min^{-1} and its amplitude was − 413 ± 61 pA (*n* = 33; Fig. 1B).

3.2. Effects of various channel blockers on the pacemaker currents of interstitial cells of Cajal

To identify the ion channel that mediates pacemaker currents, various ion channel blockers were tested. Tetrodotoxin (1 μM), a voltage-dependent Na^+ channel blocker, or nifedipine (1 μM), a voltage-dependent Ca^{2+} channel blocker, had no effect on the pacemaker currents (*n* = 4; Fig. 2A and B), and neither did tetraethylammonium chloride (2 mM) (a Ca^{2+}-activated K^+ channel blocker),

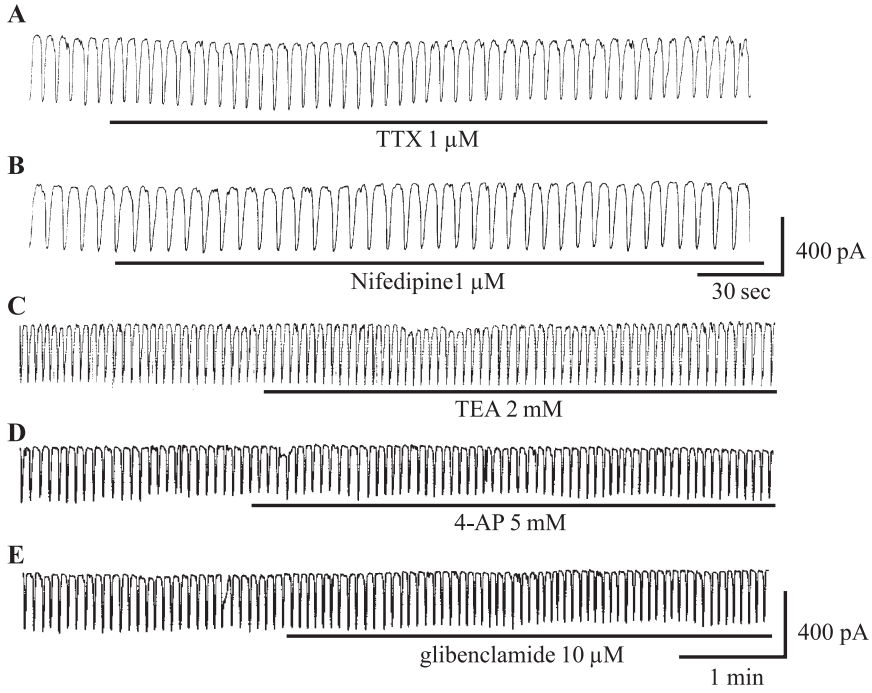

Fig. 2. Effects of various channel blockers on pacemaker currents recorded in cultured interstitial cells of Cajal from murine small intestine. Tetrodotoxin (1 μM) (A), nifedipine (1 μM) (B), tetraethylammonium chloride (2 mM) (C), 4-AP (5 mM), and glibenclamide (10 μM) had no effect on the spontaneous pacemaker currents recorded at a holding potential of − 70 mV.

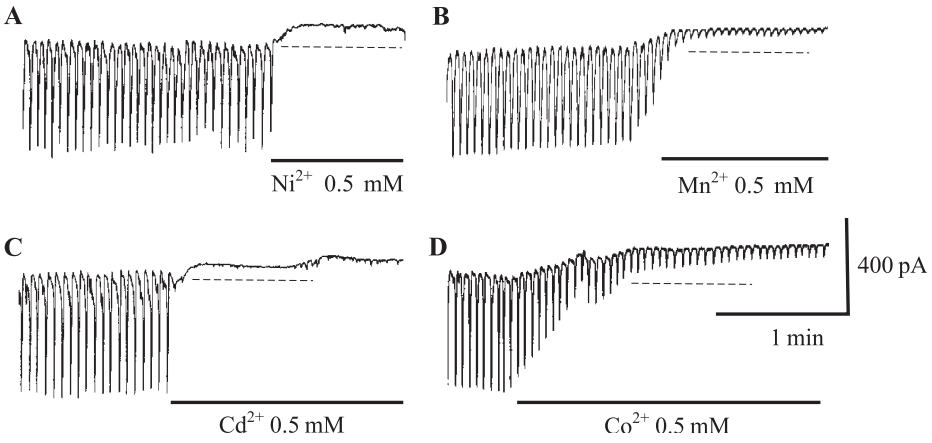

Fig. 3. Effects of divalent cations on the pacemaker currents recorded in cultured interstitial cells of Cajal from murine small intestine. Ni^{2+} (0.5 mM) (A), Mn^{2+} (0.5 mM) (B), Cd^{2+} (0.5 mM) (C), and Co^{2+} (0.5 mM) (D) all reduced the frequency and amplitude of pacemaker currents recorded at a holding potential of −70 mV. The dotted lines indicate zero current levels.

4-AP (5 mM) (a voltage-dependent K^+ channel blocker), nor glibenclamide (10 μM) (an ATP-sensitive K^+ channel blocker) ($n=5$; Fig. 2C–E).

3.3. Effects of divalent cations and non-selective cationic channel blockers on the pacemaker currents of interstitial cells of Cajal

To test the effects of divalent cations on the pacemaker currents, Ni^{2+}, Mn^{2+}, Cd^{2+}, or Co^{2+} were examined. Under a voltage clamp at a holding potential of −70 mV, Ni^{2+} (0.5 mM) ($n=4$; Fig. 3A), Mn^{2+} (0.5 mM) ($n=6$; Fig. 3B), Cd^{2+} (0.5 mM) ($n=4$; Fig. 3C), or Co^{2+} (0.5 mM) ($n=3$; Fig. 3D) inhibited the pacemaker currents, as did Gd^{3+} (10 μM) ($n=4$; Fig. 4A) or flufenamic acid (10 μM) ($n=3$; Fig. 4B) (both nonselective cation channel blockers). Under control conditions at a holding potential of −80 mV, the frequency and amplitude of the pacemaker currents were 16 ± 1.4 cycles min^{-1} and −384 ± 43 pA ($n=7$), respectively. In the presence of Gd^{3+}, the frequency and amplitude were 2 ± 0.4 cycles min^{-1} and −18 ± 7 pA, respectively, and in the presence of flufenamic acid, the frequency and amplitude were 3.5 ± 1.2 cycles min^{-1} and −24 ± 12 pA, respectively (bar graph not shown).

3.4. Effects of substance P on slow waves and pacemaker currents in interstitial cells of Cajal

Under current clamp mode, the addition of substance P (0.5 μM) produced depolarization of resting membrane potential of slow waves (Fig. 5A). Under control conditions at the current clamp mode, resting membrane potential was −59 ± 5 mV and amplitude of the slow waves was 28 ± 2 mV. In the presence of substance P, the resting membrane potential and the amplitude of slow waves were −26 ± 1.5 and 4.2 ± 1.3 mV, respectively ($n=7$, bar graph not shown). Under a voltage clamp at a holding potential of −70 mV, substance P increased the tonic inward current (resting

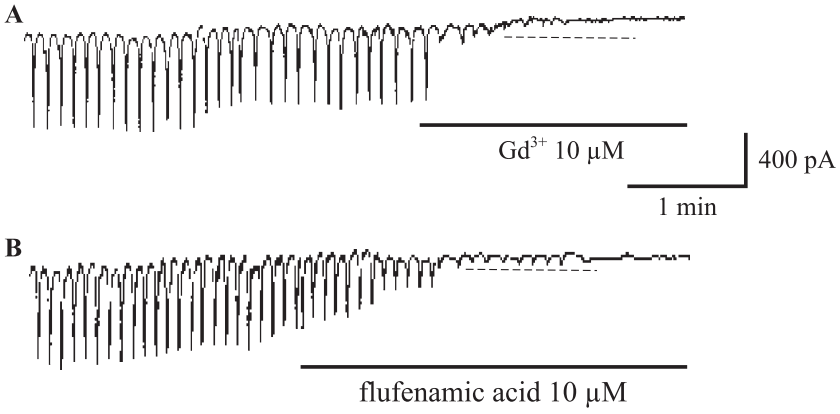

Fig. 4. Effects of nonselective cation channel blockers on pacemaker currents recorded in cultured interstitial cells of Cajal from murine small intestine. Gd^{3+} (10 μM) (A) or flufenamic acid (10 μM) (B) reduced the frequency and amplitude of pacemaker currents recorded at a holding potential of −70 mV. The dotted lines indicate zero current levels.

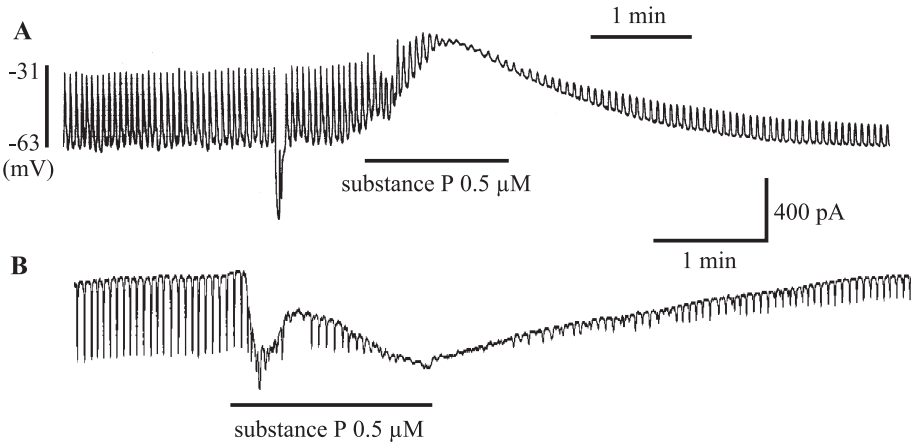

Fig. 5. Effects of substance P on slow waves and pacemaker currents recorded in cultured interstitial cells of Cajal from murine small intestine. 'A' shows the slow waves of interstitial cells of Cajal exposed to substance P ([D-Pro^2, D-Trp^{7,9}] substance P, 0.5 μM) in current clamping mode ($I=0$). Substance P induced membrane depolarization and reduced slow wave amplitude. 'B' shows the pacemaker currents of interstitial cells of Cajal treated with substance P after exposure to substance P. Substance P produced tonic inward currents and reduced the frequency and amplitude of pacemaker currents. Recordings were made at a holding potential of − 70 mV.

current) and reduced the frequency and amplitude of pacemaker current (Fig. 5B). Under control conditions, the resting current was -23 ± 9 pA, and the frequency and amplitude of the pacemaker currents were 16 ± 2 cycles min^{-1} and -420 ± 50 pA. In the presence of substance P, the resting current was -368 ± 57 pA, and the frequency and amplitude were 4 ± 2 cycles min^{-1} and -32 ± 13 pA, respectively ($n=8$, bar graph not shown).

3.5. Effects of substance P antagonist and agonist on the pacemaker currents of the interstitial cells of Cajal

To identify the receptor types of substance P, we used tachykinin NK_1 receptor antagonist and agonist. After pretreatment with tachykinin NK_1 receptor antagonist (0.5 μM, 10 min), the effects of substance P (0.5 μM) were abrogated ($n=4$, Fig. 6A). Tachykinin NK_1 receptor antagonist itself did not change the frequency and amplitude of pacemaker currents. On the other hand, tachykinin NK_1 receptor agonist (0.5 μM) produced tonic inward currents and reduced the frequency of pacemaker currents, as did substance P (Fig. 6B). Under control conditions, the resting current was -28 ± 11 pA, and the frequency and amplitude of the pacemaker current were 16 ± 2 cycles min^{-1} and -426 ± 64 pA. In the presence of tachykinin NK_1 receptor agonist, the resting current was -406 ± 48 pA, and its frequency and amplitude were 2 ± 0.9 cycles min^{-1} and -18 ± 8 pA, respectively ($n=4$, bar graph not shown).

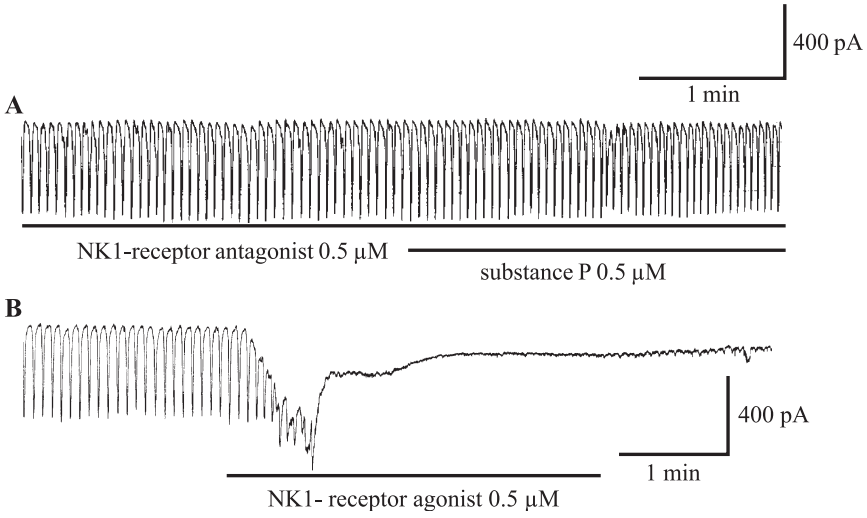

Fig. 6. Effects of substance P antagonist on substance P-induced responses, and of substance P agonist on the pacemaker currents recorded in cultured interstitial cells of Cajal from the murine small intestine. In the presence of the tachykinin NK_1 receptor antagonist ([D-Arg^1, D-Trp^{7,9}, Leu^{11}] substance P, 0.5 μM), substance P-induced responses on pacemaker currents were completely blocked (A). Whereas the tachykinin NK_1 receptor agonist ([Sar^9, Met(O$_2$)^{11}]substance P, 0.5 μM) produced tonic inward currents and reduced both the frequency and amplitude of pacemaker currents (B). Recordings were made at a holding potential of − 70 mV in cultured interstitial cells of Cajal.

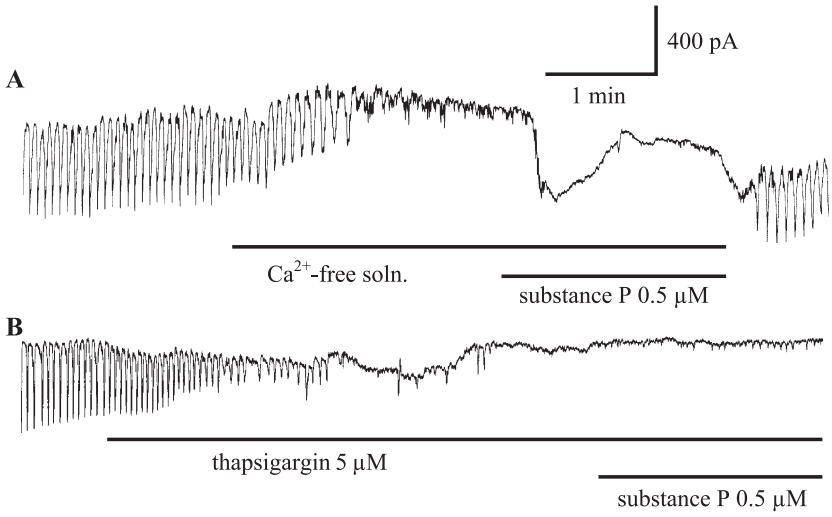

Fig. 7. Effects of an external Ca^{2+}-free medium and thapsigargin on the substance P-induced effects on the pacemaker currents of cultured interstitial cells of Cajal from the murine small intestine. The external Ca^{2+}-free solution abolished the generation of pacemaker currents at a holding potential of − 70 mV. Under this condition, substance P (0.5 μM) produced tonic inward currents (A). However, thapsigargin abolished the generation of pacemaker currents and blocked substance P-induced tonic inward currents (B).

3.6. Effects of external Ca^{2+}-free and thapsigargin on the substance P-induced currents of interstitial cells of Cajal

To investigate the roles of external and internal Ca^{2+}, the effects of substance P were investigated under external Ca^{2+} free conditions and in the presence of thapsigargin. Pacemaker currents recorded at a holding potential of − 70 mV were completely abolished in external Ca^{2+} free solution. Under this condition, substance P still produced a tonic inward current. Under normal conditions, the tonic inward current was -360 ± 48 pA ($n = 3$), and under external Ca^{2+} free conditions, this was -348 ± 36 pA ($n = 4$). However, thapsigargin blocked the tonic inward current induced by substance P. Under normal conditions, the tonic inward current was -380 ± 54 pA ($n = 3$), whereas after thapsigargin pretreatment, it was -24 ± 12 pA ($n = 4$) (Fig. 7).

3.7. Effects of reducing external Na^{+} or protein kinase C inhibitor on the substance P-induced currents of interstitial cells of Cajal

To determine the characteristic of the tonic inward currents induced by substance P, we tested the effects of substance P in the presence of 1 mM of external Na^{+}. Reducing external Na^{+} from normal to 1 mM, abolished pacemaker current. Under these conditions, substance P did not produce tonic inward currents (Fig. 8A). Under normal conditions, the tonic inward current was -368 ± 56 pA

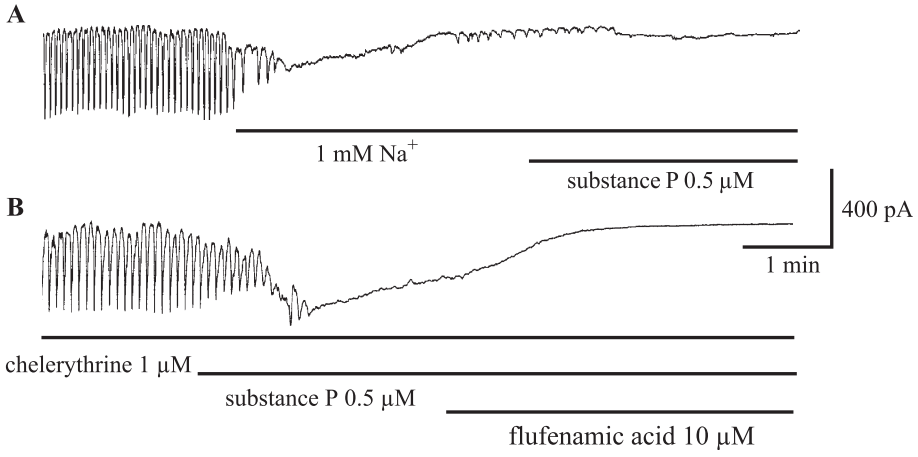

Fig. 8. Effects of reducing external Na^{+} concentrations or protein kinase C inhibitor on substance P-induced responses in cultured interstitial cells of Cajal from the murine small intestine. Reducing the external Na^{+} concentration (to 1 mM) abolished the generation of pacemaker currents, at a holding potential of − 70 mV. Under this condition, substance P did not produce tonic inward currents (A). However, in the presence of chelerythrine, substance P (0.5 μM) still produced tonic inward currents, these were blocked by flufenamic acid treatment (B).

($n=3$), and at 1 mM of Na^+ externally, the tonic inward currents was -13 ± 5 pA ($n=4$).

Protein kinase C inhibitor was used to determine whether the substance P-induced effect on pacemaker currents is mediated by the activation of protein kinase C. In the presence of chelerythrine (1 μM), a protein kinase C inhibitor, substance P continued to produce tonic inward current, which was inhibited by flufenamic acid (Fig. 8B). Under normal conditions, the tonic inward current was -345 ± 43 pA ($n=3$), and this was relatively unaffected by chelerythrine pretreatment (-330 ± 49 pA, $n=4$); moreover, chelerythrine did not change the frequency or amplitude of the pacemaker current.

4. Discussion

The present study confirms that substance P regulates intestinal motility by modulating the pacemaker currents of the interstitial cells of Cajal and that this modulation is mediated through tachykinin NK_1 receptor in a protein kinase C-independent manner.

Interstitial cells of Cajal generate spontaneous pacemaker currents that depolarize membrane, which then spreads to smooth muscle via gap junctions and results in the depolarization of smooth muscle cell membrane. This membrane depolarization leads to smooth muscle contraction by generating an action potential via the activation of voltage-dependent Ca^{2+} channels. It has been suggested that the pacemaker currents of interstitial cells of Cajal are mediated by the activation of voltage-independent nonselective cation channels (Koh et al., 1998; Thomsen et al., 1998), which allows net inward current predominantly by Na^+ under physiological condition, and which leads to excitatory action in gastrointestinal smooth muscles (Kuriyama et al., 1998).

Substance P has been reported to activate nonselective cation channels in canine colonic myocytes and in guinea pig ileal longitudinal muscles (Lee et al., 1995; Nakazawa et al., 1990). These channels are permeable to Ca^{2+} or Cs^+, whereas other divalent cations blocked them. Pharmacologically, both Gd^{3+} and flufenamate inhibited nonselective cation channels (Kuriyama et al., 1998). In the present study, tetrodotoxin, nifedipine, tetraethylammonium chloride, 4-AP, and glibenclamide were found to have no effect on pacemaker currents, but Ni^{2+}, Mn^{2+}, Cd^{2+}, and Co^{2+} blocked these currents. In addition, both Gd^{3+} and flufenamic acid, nonselective cation channel blockers, abolished pacemaker current generation, as did a reduction in external Na^+ concentrations (to 1 mM). These results strongly suggest that nonselective cationic channels are involved in generating the pacemaker currents of interstitial cells of Cajal. In the present study, substance P depolarized the membrane of interstitial cells of Cajal by increasing tonic inward currents, and these substance P-induced currents were blocked by flufenamic acid or in external 1 mM Na^+ solution, thus suggesting substance P regulates nonselective cation channels in ICCs. The excitatory actions of substance P in the gastrointestinal tract are mediated by the activation of tachykinin receptors, which are classified into 3 types, tachykinin NK_1, tachykinin NK_2, and tachykinin NK_3. Tachykinin NK_1 receptor is most potently stimulated by substance P, tachykinin NK_2 has the highest affinity for neurokinin A, and tachykinin NK_3 the highest affinity for neurokinin B. Moreover, tachykinin receptors are expressed by enteric neurons, intestinal muscles, epithelium and vasculature (Holzer and Holzer-Petsche, 2001; Olsson and Holmgren, 2001). However, tachykinin receptor distributions differ between species and in body regions. In the human esophageal body, the tachykinin NK_2 receptors contract circular smooth muscle (Huber et al., 1993). tachykinin NK_3 receptors were found to be largely confined to enteric neurons in the rat and guinea pig small intestine (Maggi, 2000). And, tachykinin NK_1 receptors were found to be expressed in several regions of interstitial cells of Cajal (Lavin et al., 1998; Vannucchi et al., 1997). In the present study, substance P-induced effects on pacemaker currents were completely blocked by the application of tachykinin NK_1 receptor antagonist. In addition, a selective tachykinin NK_1 receptor agonist was found to mimic the action of substance P. This implies that substance P coupling with the tachykinin NK_1 receptor leads to a modulation of pacemaker current.

The generation of pacemaker currents is initiated by the release of Ca^{2+} from the endoplasmic reticulum. Cyclopiazonic acid, a Ca^{2+} ATPase inhibitor in the endoplasmic reticulum, or xestospongin C, an inhibitor of inositol (1,4,5)-triphosphate receptor in the endoplasmic reticulum, abolished pacemaker current generation. However, ryanodine, a Ca^{2+}-induced Ca^{2+} release blocker in the endoplasmic reticulum, was found to have no effect on the generation of pacemaker currents (Ward et al., 2000a,b). These findings suggest that inositol (1,4,5)-triphosphate-mediated Ca^{2+} release from the endoplasmic reticulum is essentially required for pacemaker current generation. The stimulation of the tachykinin NK_1 receptor causes the activation of phospholipase C, which leads to the formation of inositol (1,4,5)-triphosphate and diacylglycerol in smooth muscles (Sanders, 1998). In this study, substance P was found to produce tonic inward pacemaker current in an external Ca^{2+}-free solution. However, substance P-induced tonic inward currents were blocked by thapsigargin, a Ca^{2+} ATPase inhibitor in the endoplasmic reticulum, suggesting that intracellular Ca^{2+} release is necessary to activate pacemaker currents. Therefore, tachykinin NK_1 receptor stimulation may increase intracellular Ca^{2+} by initiating the formation of inositol (1,4,5)-triphosphate and by increasing intracellular Ca^{2+}, leading to the activation of inward pacemaker currents. In cultured gastric interstitial cells of Cajal, phorbol 12,13-dibutyrate, a protein kinase C activator, also produced tonic inward pacemaker currents suggesting the involvement of protein kinase C in pacemaker current modulation (Kim et al., 2003). However, in the present study, chelerythrine, a

specific protein kinase C inhibitor, did not block these substance P-induced effects, suggesting that protein kinase C is not involved in tachykinin NK_1 receptor stimulation. In conclusion, our study provides evidence of functional tachykinin NK_1 receptor on the interstitial cells of Cajal of the murine small intestine. Substance P depolarized the membrane and increased tonic inward currents, which were activated by Ca^{2+} release from internal stores induced by tachykinin NK_1 receptor activation. In addition, substance P was found to activate nonselective cation channels in a protein kinase C-independent manner. Thus, tachykinin NK_1 receptor may play a central role in the regulation the rhythm and contraction of intestinal smooth muscles by acting on the interstitial cells of Cajal.

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