Invited Review**Mechanism of abnormal intestinal motility in inflammatory bowel disease: how smooth muscle contraction is reduced?**Takashi OHAMA^{1*}, Masatoshi HORI¹ and Hiroshi OZAKI¹¹*Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo*

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Abstract

Intestinal inflammation alters the contractile activity of intestinal smooth muscle. Motility disorders of the gastrointestinal tract are clinically important symptoms, because they are often associated with severe interstitial inflammation. In addition, the motility disorders secondarily induce abnormal growth of the intestinal flora, and the resulting disturbance of this flora aggravates the pathogenesis of mucosal inflammation. This in turn aggravates the intestinal dysmotility; *i.e.*, it is an inflammatory spiral. Therefore, it is important to elucidate the mechanisms involved in the changes in motor function which occur in intestinal inflammation. Recent studies have revealed several molecular mechanisms responsible for the decreased motility which occurs in an inflamed gastrointestinal tract. In the present review, we discuss the functional failure of smooth muscle cells, including changes in the activity of muscarinic receptors, ion channels and the endogenous myosin phosphatase inhibitor CPI-17.

Key words: intestinal inflammation, smooth muscle, contraction, CPI-17

Motility disorders in intestinal inflammation

Motility disorders of the gastrointestinal tract are extremely important clinically because they can lead to systemic disease. In humans and in animal models, intestinal inflammation results in the disturbance of motility, which may reflect changes in smooth muscle function and/or the enteric nervous system (Vermillion *et al.*, 1993; Vrees *et al.*, 2002). Both increased and decreased smooth muscle contractility has been observed in intestinal inflammation. In a model of nematode infection-induced gut inflammation, such as *Trichinella spiralis* infection-induced gut inflammation, smooth muscle contractility is increased (Vermillion and Collins, 1988; Blennerhassett *et al.*, 1992). On the other hand, smooth muscle contractility is decreased

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in the intestinal inflammation induced by trinitrobenzene sulphonic acid (TNBS), surgical manipulation, experimental obstruction, hemorrhagic shock, or peritonitis (Kalff *et al.*, 1998; Moreels *et al.*, 2001; Koyluoglu *et al.*, 2002; Hierholzer *et al.*, 2004; Kinoshita *et al.*, 2006; Kiyosue *et al.*, 2006; Won *et al.*, 2006; Kinoshita *et al.*, 2007; Ohama *et al.*, 2007b). These differences in contractile responses may be due to differences in cytokine profiles. Reports from the Collins group (Akiho *et al.*, 2002; Akiho *et al.*, 2005a; Akiho *et al.*, 2005b) have suggested that nematode-induced hypercontractility is mediated by an increase in prostaglandin E₂ (PGE₂) levels after induction of the expression of Th2 cytokines such as interleukin (IL)-4 and IL-13. On the other hand, reports suggest that TNBS-induced gut inflammation is mediated mainly by Th1 cytokines such as IL-1 β , tumor necrosis factor- α (TNF- α) and IL-12 (Neurath *et al.*, 1995; Kinoshita *et al.*, 2006; Kiyosue *et al.*, 2006; Ohama *et al.*, 2007b). In this review, we focused on the molecular mechanisms that are responsible for the decreased motility of the inflamed intestine. Nematode-induced hyper-contraction is discussed in great detail in a previous review (Khan and Collins, 2006).

Muscarinic receptor activity is changed in intestinal inflammation

Various contractile factors, such as acetylcholine, 5-hydroxytryptamine (5-HT), substance P, motilin and prostaglandin F_{2 α} (PGF_{2 α}), induce intestinal smooth muscle cell contraction. Acetylcholine is thought to be the principal neurotransmitter involved in intestinal smooth muscle contraction. Intestinal smooth muscle cells express 5 isoforms of muscarinic receptor: M₁ to M₅ (Preiksaitis *et al.*, 2000; Wang *et al.*, 2000). M₂ and M₃ receptors are thought to be involved in contractile activity (Sawyer and Ehlert, 1999; Stengel *et al.*, 2000; Matsui *et al.*, 2002; Stengel *et al.*, 2002). It has been suggested that the M₂ receptor supports M₃-mediated activity and indirectly contributes to smooth muscle contraction. However, more recent reports indicate that the M₂ receptor can directly activate L-type Ca²⁺ channels and nonselective cation channels via c-src, phosphoinositide 3-kinase (PI3K) and protein kinase C (PKC), and contribute to intestinal smooth muscle contraction (Inoue and Kuriyama, 1991; Wan *et al.*, 1999; Jin *et al.*, 2002).

In an acetic acid-induced dog ileitis model, acetylcholine-induced contraction of circular smooth muscle is decreased, but contraction of longitudinal smooth muscle is unchanged (Shi and Sarna, 1999). In a TNBS-induced guinea-pig ileitis model, although the contractile force of the circular smooth muscle in response to acetylcholine is decreased, the contractile force of the longitudinal smooth muscle is increased (Martinolle *et al.*, 1997). These differences in responses to inflammation between circular and longitudinal smooth muscle may be due to the degree of dependence on M₂ receptors for muscarinic agonist-induced contraction. That is, circular smooth muscle contraction may be mediated mainly by M₃ receptors, whereas the major part of the longitudinal smooth muscle contraction may be mediated by M₂ receptors. When intestinal inflammation shifts the balance of involvement of M₂ and M₃ receptors in smooth muscle contraction toward predominance of M₂ receptors, the effect of this change in balance is limited to the longitudinal smooth muscle, because M₂ receptors are not involved in circular smooth muscle contraction (Shi and Sarna, 1999; Jadcherla, 2002).

Studies using muscarinic receptor knock-out mice indicate that in carbachol-induced contraction of the longitudinal smooth muscle of the mouse ileum, the contributions of M_2 and M_3 receptors are about 25% and 75%, respectively (Matsui *et al.*, 2002). However, other studies indicate that in carbachol-induced ileal smooth muscle contraction, only low-dose induced contraction involves M_2 receptors (Unno *et al.*, 2005), and that although M_2 receptors are involved in sustained carbachol-induced contraction of longitudinal smooth muscle of the mouse ileum, they are not involved in transient contractions (Takeuchi *et al.*, 2006). These findings suggest that the observed differences in contractile responses to inflammation between circular and longitudinal smooth muscle may not be fully explained by the degree of dependence on different muscarinic receptor isoforms. Further studies are needed to clarify this issue. Moreover, some reports indicate that TNBS-induced inflammation decreases muscarinic receptor-mediated contraction of longitudinal smooth muscle (Moreels *et al.*, 2001; Ohama *et al.*, 2007b).

L-type Ca^{2+} channel activity is suppressed in intestinal inflammation

Disturbance of L-type Ca^{2+} channel activity has been reported as one of the causes of decreased intestinal motility (Akbarali *et al.*, 2000; Shi *et al.*, 2000; Liu *et al.*, 2001; Kinoshita *et al.*, 2003; Shi *et al.*, 2005). There are 2 splicing variants of the α_{1c} subunit of L-type Ca^{2+} channels (Tanabe *et al.*, 1987; Koch *et al.*, 1989; Holm *et al.*, 2000; Soldatov, 2003), and discrete promoters mediate expression of these 2 isoforms (Saada *et al.*, 2003). Intestinal smooth muscle cells express both of these isoforms (Kang *et al.*, 2004). Akbarali *et al.* (2000) were the first to investigate the activity of Ca^{2+} channels in a mouse model of experimental colitis. They induced intestinal inflammation using dextran sulphate sodium (DSS), and examined changes in voltage-dependent L-type Ca^{2+} currents using whole-cell patch-clamp techniques. They found that inward Ca^{2+} currents were markedly suppressed in the inflamed colon. Liu *et al.* (2001) found that expression of the α_{1c} subunit of L-type Ca^{2+} channels was significantly reduced in intestinal inflammation induced by acetic acid and ethanol, and that this decreased expression was associated with suppression of Ca^{2+} channel currents in patch-clamped cells. Other groups have reported that in the DSS-induced mouse colitis model (Kang *et al.*, 2004) and the TNBS-induced rat colitis model (Kinoshita *et al.*, 2003), although the activity of L-type Ca^{2+} channels was decreased, expression of the α_{1c} subunit remained unchanged. The difference in these findings may be due to differences in the animal species used in those studies and/or differences in the methods used to induce inflammation. As for the mechanism of decreased protein expression of the α_{1c} subunit, in a study by Shi *et al.* (2005) using human colonic smooth muscle cells, TNF- α -induced activation of nuclear factor- κ B (NF- κ B) resulted in suppression of the mRNA expression of the α_{1c} subunit. They also found that protein expression of the α_{1c} subunit was suppressed 6 hours after induction of TNF- α . However, in very recent studies using smooth muscle-specific L-type Ca^{2+} channel knockout mice, the half-life of the α_{1c} subunit was about 14 days (Wegener *et al.*, 2006). This suggests that suppression of mRNA expression of the α_{1c} subunit is not the only mechanism involved in decreased α_{1c} subunit protein expression, and that mechanisms such as accelerated protein degradation and

decreased mRNA stability are involved. Further studies are needed to clarify this issue.

Wegner *et al.* (2006) reported that in the mouse colon, smooth muscle-specific deletion of L-type Ca^{2+} channels results in 50% suppression of carbachol-induced contraction. They also found that deletion of L-type Ca^{2+} channels did not affect carbachol-induced contraction of the ileum, because store-operated Ca^{2+} channels compensated for the decreased activity of L-type Ca^{2+} channels. It appears that suppression of L-type Ca^{2+} channel activity is involved in the decreased contraction of colonic smooth muscle cells, but not in the decreased contraction of ileal smooth muscle cells.

K_{ATP} channel activity is increased in intestinal inflammation

Tissue hypoxia resulting from ischemia is usually accompanied by inflammation and usually leads to cell damage. However, some cells tolerate extended periods of hypoxia, and an understanding of the mechanisms underlying this tolerance of oxygen depletion is pivotal for development of strategies for protecting cells from the consequences of hypoxic insults. Activation of ATP-sensitive K^+ (K_{ATP}) channels by processes associated with energy deprivation is an important mediator in counteracting membrane depolarization of damaged cells, especially in the heart and brain during tissue hypoxia (Ashcroft and Gribble, 2000; Melamed-Frank *et al.*, 2002; Unno *et al.*, 2005).

The K_{ATP} channel is a complex composed of inwardly rectifying, pore-forming subunits (Kir 6.1 and Kir 6.2) and the sulfonylurea receptors (SUR1 and SUR2). In intestinal smooth muscle, these channels are important for regulation of cell excitability. Jin *et al.* (2004) examined the molecular composition of the K_{ATP} channel in mouse colonic smooth muscle, and examined its activity in DSS-induced experimental colitis. Levcromakalim (an activator of K_{ATP} channels) - induced currents in whole-cell recordings were significantly larger in inflamed cells than in non-inflamed cells. In cell-attached patch recordings of single-channel events, levcromakalim increased the bursting duration of inflamed cells. In cells that co-express Kir 6.1 and Kir 6.2, Kir 6.1 is localized in the plasma membrane, whereas Kir 6.2 is mainly detected in the cytosol. Polymerase chain reaction (PCR) analysis indicates that Kir 6.1 mRNA expression is up-regulated after inflammation, suggesting that the decreased motility of colonic smooth muscle during inflammation is associated with changes in the transcriptional regulation of K_{ATP} channel components.

CPI-17 is down-regulated in intestinal inflammation

The contractile system of smooth muscle cells is basically regulated by myosin light chain (MLC) phosphorylation, which is driven by the balance between myosin light chain kinase (MLCK) activity and myosin phosphatase activity (Kamm and Stull, 1989). During receptor agonist-induced contractions, contractile elements are sensitized to Ca^{2+} , and this induces greater MLC phosphorylation and greater force at a given cytosolic Ca^{2+} level (Hori and Karaki, 1998; Somlyo and Somlyo, 2000; Pfitzer, 2001). Recent studies indicate that RhoA (a small GTP-binding protein) and RhoA-dependent coiled serine/threonine kinases (ROCKs) play important

roles in this Ca^{2+} sensitization. Activated RhoA (RhoA-GTP) activates ROCKs, which in turn phosphorylate a noncatalytic subunit of myosin phosphatase (MYPT1), thereby inactivating myosin phosphatase. On the other hand, receptor stimulation also activates PKC. The activated PKC phosphorylates PKC-potentiated phosphatase inhibitor protein-17 (CPI-17) (Eto *et al.*, 1997; Senba *et al.*, 1999), which in turn inactivates myosin phosphatase. As a result, MLCK-induced MLC phosphorylation is augmented, thereby inducing greater contractions at a given cytosolic Ca^{2+} level (see Fig. 1).

CPI-17 was first isolated from pig aorta, and was identified as an endogenous inhibitor of serine/threonine protein phosphatase (Eto *et al.*, 1995; Eto *et al.*, 1997). Activation of CPI-17 is regulated by phosphorylation of Thr-38, and several kinases are capable of doing this, including not only PKC but also ROCKs, ZIP-kinase, PKN, and integrin-linked kinase (Hamaguchi *et al.*, 2000; Kitazawa *et al.*, 2000; Koyama *et al.*, 2000; MacDonald *et al.*, 2001; Ohama *et al.*, 2003). Activated CPI-17 directly binds to the catalytic subunit of myosin phosphatase (PP1c δ), thereby inhibiting myosin phosphatase activity and increasing the relative amount of phosphorylated MLC and augmenting smooth muscle contraction (Eto *et al.*, 2004). CPI-17 activation is thought to be important for intestinal smooth muscle contraction, because blocking CPI-17 activity completely abolishes Ca^{2+} -sensitization induced by a muscarinic agonist plus GTP (Ohama *et al.*, 2007b). Various receptor agonists, such as cholinergic agents, histamine, endothelin and angiotensin II, activate CPI-17 in tandem with induction of myosin phosphorylation and smooth muscle contraction at certain intracellular Ca^{2+} concentrations (Kitazawa *et al.*, 2000; Kitazawa *et al.*, 2003; Ohama *et al.*, 2003).

In a previous study using a tissue culture system, we found that long-term treatment of intestinal smooth muscle with IL-1 β attenuated smooth muscle contraction (Ohama *et al.*, 2003). When ileal smooth muscle strips were cultured with IL-1 β , contractions elicited by high K^+ or carbachol were inhibited in a time-dependent manner. IL-1 β inhibited the carbachol-induced contractions more strongly than it inhibited the high K^+ -induced contractions, and MLC phosphorylation levels decreased. In α -toxin-permeabilized ileal muscle, carbachol plus GTP or GTP- γ S increased the Ca^{2+} sensitivity of contractile elements, and this G-protein-coupled Ca^{2+} sensitization was significantly reduced in the IL-1 β -treated ileum. Among the functional proteins involved in smooth muscle Ca^{2+} sensitization, CPI-17 expression was reduced after culturing with IL-1 β , whereas the expression of other regulatory proteins, such as RhoA, ROCK-I, ROCK-II, MYPT-1, MLCK and myosin phosphatase (PP1), was unchanged. The level of CPI-17 phosphorylation induced by carbachol was low, which is consistent with the decrease in CPI-17 expression caused by IL-1 β treatment. A subsequent study using TNF- α knockout mice and IL-1 α/β knockout mice revealed that IL-1 β -induced down-regulation of CPI-17 is mediated by an IL-1 β induced increase in TNF- α production, and that TNF- α acts as a crucial mediator of the decrease in CPI-17 expression (Ohama *et al.*, 2007b). In that study, down-regulation of CPI-17 was observed in an *in vivo* model in which ileitis was induced by TNBS. We have observed decreased CPI-17 protein expression in smooth muscle from ulcerative colitis patients and IL-10 knockout mice used as a spontaneous colitis model (our unpublished observations). Thus, the available evidence suggests that decreased CPI-17 protein expression during inflammation is associated with decreased motility of intestinal smooth muscle.

Fig. 1. Mechanism of smooth muscle contraction and the impairment of contractility in intestinal inflammation (details see text).

In addition to the long-term effects of IL-1 β , it has been reported that short-term activity of IL-1 β inhibits smooth muscle contractility by modulating the release of acetylcholine and norepinephrine, which are neuromediators in the rat myenteric plexus (Hurst and Collins, 1993; Main *et al.*, 1993), but these inhibitory effects disappear within a few hours.

Other mechanisms

Various histological abnormalities have been observed in patients with inflammatory bowel disease (IBD) and in experimental models of intestinal inflammation, including damage to the nervous system (Jacobson *et al.*, 1995; Sanovic *et al.*, 1999; Galeazzi *et al.*, 2000; Lin *et al.*, 2005), disruption of the interstitial cells of Cajal (ICC) network (Lu *et al.*, 1997; Rasmussen *et al.*, 1997; Der *et al.*, 2000; Chang *et al.*, 2001; Faussone-Pellegrini *et al.*, 2002; Porcher *et al.*, 2002), and hyperplasia and hypertrophy of smooth muscle cells (Morson, 1968; Blennerhassett *et al.*, 1992). Intestinal smooth muscle cells receive neural input from axons that originate within the intestine, and disruption of the intrinsic nerve has been shown to lead to proliferation of smooth muscle cells (Lourenssen *et al.*, 2005). As an alternative mechanism, in studies using cultured intestinal smooth muscle cells, IL-1 β has been shown to directly affect smooth muscle cells and promote their proliferative activity (Owens and Grisham, 1993; Graham *et al.*, 1996; Hogaboam *et al.*, 1997). Thus, it appears that IL-1 β is involved in hyperplasia of the smooth muscle layer.

However, in a recent study using a tissue culture system, we found that IL-1 β acts indirectly as an anti-proliferative mediator of intestinal smooth muscle cells, by inducing production of PGE₂ and nitric oxide (NO) by resident macrophages in ileal smooth muscle tissue (Ohama *et al.*, 2007a). Disruption of the ICC network in an inflamed intestine may cause a decrease in membrane potential of smooth muscle cells, because studies suggest that the ICC network mediates hyperpolarization of smooth muscle cells (Cohen *et al.*, 1986; Lu *et al.*, 1997). Several studies indicate that immunological events in intestinal muscle during surgical manipulation closely correlate with abnormal intestinal motility. Bauer group found that surgical manipulation induced expression of TNF- α , cyclo-oxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in gastric and colonic muscularis, leading to decreased smooth muscle contractility (Schwarz *et al.*, 2001; Schwarz *et al.*, 2004).

Conclusions

In the present review, we discussed the mechanisms of the decreased smooth muscle contractility observed in intestinal inflammation, in which histological and functional abnormalities are involved. At present, 3 main types of functional failure are thought to be involved in this decreased contractility: 1) changes in activity of muscarinic receptors, 2) changes in activity of ion channels, and 3) changes in activity of the MLC phosphatase inhibitor CPI-17 (see Fig. 1). Several studies indicate that there are regional differences in responses to inflammatory stimuli; *e.g.*, differences between the ileum and colon. Differences in the inflammatory stimuli used in various studies have also led to differences in the observed effects of inflammation on smooth muscle contractility. The available evidence suggests that the mechanism of motility dysfunction depends on the intestinal region, inflammatory stimuli, and the stage of inflammation.

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