Invited Review

Muscularis mucosae — the forgotten sibling

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Abstract

Lamina muscularis mucosae sitting beneath mucosal surface of the digestive tract has received little attention to date compared with external smooth muscle layers. Motor activity of the muscularis mucosae shows a great regional and species difference. Autonomic innervation profile is also different from esophagus to colon or between animal species. Intracellular transduction mechanisms for motor activity of the muscularis mucosae are also different from those of external longitudinal and circular muscles or from vascular and airway smooth muscles. Since the submucosal area is a major source for eicosanoid production, abnormality of muscularis mucosae motor activity may link with abnormality of mucosal absorption and secretion functions. Inflammatory bowel diseases such as diarrhea, irritable bowel syndrome and Crohn's disease accompanied with altered motor activity of the muscularis mucosae. Much attention should be attracted to the human muscularis mucosae as a new therapeutic target for inflammatory bowel diseases.

Key words: muscularis mucosae, motor activity, autonomic innervation, digestive tract, inflammatory bowel disease

Introduction

Lamina muscularis mucosae is a thin layer of smooth muscles located beneath luminal mucosa throughout the digestive tract from esophagus to colon. Their types of smooth muscles are different between esophagus and gastro-intestine. Esophageal muscularis mucosae is composed of only longitudinal smooth muscles, while gastric and intestinal muscularis mucosae are composed of outer longitudinal and inner circular smooth muscles (Freeman and Bracegirdle, 1967). Muscularis mucosae is absent in anal canal. Despite widespread distribution of muscularis mucosae in the digestive tract, the physiological role for gut function has, until recently, been poorly defined, especially when compared with its more illustrious sibling, external smooth muscle layers (Bülbring *et al.*, 1981, Kuriyama *et al.*, 1998, Grundy *et al.*, 2006). However, the muscularis mucosae probably has great influences on the absorptive and secretory functions of epithelium because the mucosa sits on this muscle layer and because

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fingers of the muscularis mucosae project into the pits and villi of the mucosa (Greenwood and Davison, 1987). The early physiological studies by King and his colleagues (King and Arnold, 1922; King *et al.*, 1922; King and Church, 1923; King and Robinson, 1945; King *et al.*, 1947) had already appeared unique characteristics of this smooth muscle in different from external smooth muscles. They presented evidence that the muscularis mucosae would imply movement of the mucosa and the villi through excitatory cholinergic and adrenergic nerves but not inhibitory innervations. In 1960s–1970s, histochemical studies had revealed the distribution of adrenergic, cholinergic and peptidergic nerve fibers in the muscularis mucosae (Furness and Costa, 1980). Since 1977, we have reported their pharmacological characteristics using the muscularis mucosae isolated from various regions of the digestive tract. Here we summarize histochemical, physiological and pharmacological characteristics of the muscularis mucosae and their clinical implications.

Esophageal muscularis mucosae

Autonomic innervations

Esophageal muscularis mucosae is composed of abundant longitudinal smooth muscles throughout esophageal body in all animal species including human (Freeman and Bracegirdle, 1967). The longitudinal muscularis mucosae is innervated by postganglionic excitatory cholinergic nerves via myenteric and submucous plexus from vagal nerve in all animal species (Thomas and Trounce, 1960; Bartlet, 1968a; Kamikawa and Shimo, 1979a; Bieger and Triggle, 1985; Christensen *et al.*, 1987a; Surprenant, 1994; Kerr *et al.*, 1995; Kerr, 2002). Adrenergic innervation to esophageal muscularis mucosae via thoracic sympathetic nerves is sparse, but the density is different between animal species. The muscularis mucosae of the cat or rhesus monkey esophagus was innervated with rich adrenergic nerve fibers (Baumgarten and Lange, 1969), whereas that of the guinea-pig or rabbit esophagus was innervated with less nerve fibers (Nishimura and Takasu, 1969; Kamikawa and Shimo, 1979a). The muscularis mucosae is also innervated by polypeptide-containing nerve fibers. Neuropeptides such as tachykinins, vasoactive intestinal peptide (VIP), calcitonin-gene related peptide (CGRP), enkephalin, galanin, neuropeptide Y (NPY) and somatostatin are thought to be functioned as an afferent neurotransmitter (Uddman *et al.*, 1978; Leander *et al.*, 1982; Domoto *et al.*, 1983; Keast *et al.*, 1985; Christensen *et al.*, 1987b; Singaram *et al.*, 1991), because the muscularis mucosae produced neither non-cholinergic nor non-adrenergic neurogenic responses (Kamikawa and Shimo, 1979a; Robotham *et al.*, 1985). Their distributions, however, are different from species to species and between upper and lower esophagus (Uddman *et al.*, 1980; Singaram *et al.*, 1991; Holzer and Holzer-Petsche, 1997).

(1) Human: The muscularis mucosae isolated from the human fetal esophagus usually showed spontaneous activity. Exogenously applied acetylcholine or physostigmine produced an atropine-sensitive sustained contraction of the muscularis mucosae, while pilocarpine gave a contraction accompanied by an increase in spontaneous activities (Hughes, 1957; Christensen, 1975). These raise the possibility that the muscularis mucosae is innervated by excitatory cholinergic nerves. Exogenously applied adrenaline inhibited spontaneous activities of the

muscularis mucosae, but failed to relax the acetylcholine-induced contraction. Since tyrosine hydroxylase immunoreactive neuronal cell bodies were found in Meissner's plexus of the esophagus (Wakabayashi *et al.*, 1989), these indicate that adrenergic nerves might inhibit spontaneous motility via the inhibition of cholinergic neurotransmission. A dense plexus of VIP-, NPY-, CGRP- and galanin-immunoreactive nerve fibers was observed in the human esophageal muscularis mucosae (Keast *et al.*, 1985; Wattchow *et al.*, 1987; Singaram *et al.*, 1991). The physiological relevance of these peptidergic nerves remains unclear.

(2) Guinea-Pig: The longitudinal muscularis mucosae isolated from the guinea-pig esophagus usually showed neither resting tone nor spontaneous activity (Bailey, 1965). We have observed that a ganglionic stimulant nicotine produced a transient contraction of the muscularis mucosae which was abolished by the pretreatment with tetrodotoxin, hexamethonium or atropine. Electrical field stimulation of the muscularis mucosae evoked a twitch-like contraction which was enhanced with physostigmine but abolished with atropine or tetrodotoxin (Kamikawa and Shimo, 1979a; Kamikawa and Shimo, 1983a; Kamikawa *et al.*, 1982). By using the vagal nerve-attached muscularis mucosae preparation, we and other investigators have shown that vagal stimulation produced a transient contraction of the muscularis mucosae which was also abolished by the pretreatment with tetrodotoxin, hexamethonium, or atropine (Bartlet, 1968a; Kamikawa and Shimo, 1979a; Kerr *et al.*, 1995). These observations reveal that the esophageal muscularis mucosae is innervated by excitatory vagal (parasympathetic) nerve-submucous plexus-muscarinic receptor pathway. Ohkawa (1980) had also reported that the circular muscularis mucosae of the guinea-pig gastro-esophageal junction was innervated by excitatory cholinergic nerves. We have observed that only a few but fine catecholamine-containing nerve fibers were seen to be in close contact with bundles of smooth muscle in the lamina muscularis mucosae of the guinea-pig esophagus (Kamikawa and Shimo, 1979a). The guinea-pig esophageal muscularis mucosae had postjunctional excitatory α_1- and inhibitory β_1-adrenoceptors, but not α_2-adrenoceptors (Kamikawa *et al.*, 1982; Uchida, 1983; Uchida *et al.*, 1983; Kamikawa and Shimo, 1987; Horinouchi *et al.*, 2003). In the presence of atropine, electrical field stimulation of the histamine-contracted muscularis mucosae evoked a weak relaxation which was abolished by the pretreatment with tetrodotoxin, guanethidine or propranolol (Kamikawa and Shimo, 1979a). Our findings indicate that the muscularis mucosae of the guinea-pig esophagus is sparsely innervated by inhibitory adrenergic nerves. Noradrenaline released from adrenergic nerve terminals might relax the muscularis mucosae through postjunctional β_1-adrenoceptors. Furness *et al.* (1994) have demonstrated the distribution of nitric oxide synthase-containing nerve fibers in the muscularis mucosae of the guinea-pig esophagus. But we could not observe the nitric oxide-mediated response to electrical field stimulation (Kamikawa and Shimo, 1979a). Furthermore, neither nitroprusside nor dibutyryl cyclic GMP relaxed the esophageal muscularis mucosae (Kamikawa and Shimo, 1987). These indicate that nitrergic nerve-cyclic GMP pathway has no role for motor function of the muscularis mucosae. Tachykinin-like immunoreactivity has been detected in sensory nerves of the guinea-pig esophagus (Hua *et al.*, 1985). Kerr *et al.* (1995) have demonstrated that vagal nerve stimulation of the guinea-pig esophagus evoked a triphasic contractile response. The third response to vagal stimulation was abolished by the pretreatment with tetrodotoxin or

capsaicin, but not with hexamethonium or tubocurarine, indicating the mediation by the release of substance P-like neuropeptide from sensory nerve endings. But we could not observe any non-cholinergic excitatory response of the esophageal muscularis mucosae (Kamikawa and Shimo, 1979a). The discrepancy may be due to different experimental conditions, such as whole esophagus preparation or stimulating frequency. Electrical stimulation with high frequency and long pulse duration of smooth muscle preparations may activate non-neuronal structures. Thus, peptidergic nerves innervating to the muscularis mucosae function as an afferent sensory nerve, but are negligible for efferent motor activity.

(3) Rat: The muscularis mucosae isolated from the rat esophagus showed neither intrinsic tone nor spontaneous activity. Electrical field stimulation of the muscularis mucosae produced a sustained contraction, which was abolished by the pretreatment with tetrodotoxin or scopolamine, but enhanced with BW284C51, a selective acetylcholinesterase inhibitor (Hughes, 1955; Bieger and Triggle, 1985). Storr *et al.* (2001) have also demonstrated that vagal nerve stimulation of the whole esophagus preparation can produce a contraction of the muscularis mucosae which was abolished by the pretreatment with hexamethonium or atropine. These indicate that rat esophageal muscularis mucosae is innervated by excitatory vagal pre- and post-ganglionic cholinergic nerves. Buckner and Christopherson (1974) have indicated that postjunctional α-adrenoceptors are not present in the esophageal muscularis mucosae. The catecholamine-induced relaxation of the rat esophageal muscularis mucosae was predominantly mediated by postjunctional β_3-adrenoceptors (De Boer *et al.*, 1993; De Boer *et al.*, 1995; Oostendorp *et al.*, 2004). Electrical field stimulation of the muscularis mucosae pre-contracted with muscarinic agonist produced the tetrodotoxin-sensitive relaxations which were inhibited by the pretreatment with guanethidine, particularly in the distal part of the thoracic esophagus, but not cervical or proximal parts (Will *et al.*, 1990). The rat esophageal muscularis mucosae may be sparsely innervated by inhibitory adrenergic nerves. The rat esophageal muscularis mucosae is known to receive a nitrergic innervation (Wörl *et al.*, 1994; Neuhuber *et al.*, 1994). The electrically-evoked relaxation of the pre-contracted muscularis mucosae from the cervical esophagus was inhibited by the pretreatment with tetrodotoxin but enhanced with L-arginine. The enhancing effect of L-arginine was blocked by N^G-monomethyl-L-arginine, and was not mimicked by the D-arginine treatment (Will *et al.*, 1990). Thus, the rat cervical esophageal muscularis mucosae may be innervated partly by inhibitory nitrergic nerves. In the rat vagal nerve-attached whole esophagus preparation, vagal stimulation evoked a non-cholinergic and non-adrenergic relaxation of the inner muscularis mucosae (Akabarali *et al.*, 1986). The inhibitory response may be mediated by the antidromic activation of sensory nerves. Several neuropeptides are known to be present in vagal sensory nerve fibers, but exact neurotransmitter responsible for the inhibitory response is still unknown.

(4) Rabbit: The muscularis mucosae isolated from the rabbit thoracic esophagus, but not cervical esophagus, usually showed spontaneous motor activity and resting tone. Electrical field stimulation or treatments with physostigmine or nicotine produced a contraction of the muscularis mucosae which was abolished by the pretreatment with tetrodotoxin or atropine. None of inhibitory neurogenic response was observed (Hughes, 1955; Percy *et al.*, 1997). The rabbit esophageal muscularis mucosae seems to be innervated solely by excitatory cholinergic nerves.

(5) Opossum: The muscularis mucosae isolated from the opossum esophagus, except of the proximal region, exhibited spontaneous motor activity. Electrical field stimulation of the muscularis mucosae produced a biphasic contraction, consisting of an initial phasic contraction followed by a sustained contraction. The initial contraction was abolished by the pretreatment with tetrodotoxin or atropine, but enhanced with physostigmine, while the following contraction was abolished with tetrodotoxin, [D-Pro^{2}, D-Trp^{7,9}] substance P or capsaicin (Christensen and Percy, 1984; Domoto *et al.*, 1983; Robotham *et al.*, 1985). These suggest that the opossum esophageal muscularis mucosae might receive both cholinergic and tachykininergic excitatory innervation. On the proximal esophageal muscularis mucosae, noradrenaline produced neither contraction nor relaxation, but diminished the amplitude of cholinergically-mediated contraction without affecting the resting tone. The diminishing effect was antagonized with yohimbine, but not with propranolol (Christensen and Percy, 1984). These observations indicate that adrenergic nerves can modulate the motility of the muscularis mucosae via prejunctional inhibitory α_2-adrenoceptors located on excitatory cholinergic nerves. In contrast to proximal regions, noradrenaline produced a biphasic response of the distal esophageal muscularis mucosae, consisting of an initial contraction followed by a relaxation. The initial contraction was blocked by the pretreatment with phentolamine but following relaxation was blocked with propranolol. Noradrenaline also caused a minimal depression of the electrically-evoked cholinergic contraction which was antagonized with propranolol. Clonidine had no effect on the neurogenic contraction (Christensen and Percy, 1984). Adrenergic nerves innervating to the distal esophageal muscularis mucosae seem to have a direct influence on smooth muscles.

(6) Cat: The muscularis mucosae isolated from the cat thoracic esophagus spontaneously developed motor activity. Electrical field stimulation or treatment with physostigmine or nicotine of the muscularis mucosae produced a contraction which was abolished by the pretreatment with tetrodotoxin or atropine (Hughes, 1955; Christensen and Percy, 1984). These observations suggest that the cat esophageal muscularis mucosae is mostly innervated by excitatory postganglionic cholinergic nerves. The muscularis mucosae responded to noradrenaline with a contraction which was antagonized with phentolamine (Christensen and Percy, 1984). In the presence of phentolamine, noradrenaline produced a relaxation which was abolished with propranolol. Noradrenaline also inhibited the electrically-induced and cholinergically-mediated contraction of the muscularis mucosae which was antagonized with yohimbine or propranolol. It seems likely that adrenergic nerves innervating the muscularis mucosae of the cat esophagus regulate motor activity via both pre- and post-junctional adrenoceptors. Nicotine caused a relaxation of the circular muscularis mucosae from the lower esophagus. The relaxation was abolished by the pretreatment with tetrodotoxin or N$^{\omega}$-nitro-L-arginine, but not with guanethidine or propranolol (Dobreva *et al.*, 1994). The circular muscularis mucosae of the cat lower esophagus seems to be innervated by inhibitory nitrergic nerves.

(7) Dog: The muscularis mucosae isolated from the dog esophagus showed spontaneous motor activity. Electrical field stimulation of the muscularis mucosae evoked a contraction which was abolished by the pretreatment with tetrodotoxin or atropine (Christensen and Percy, 1984). Dog esophageal muscularis mucosae seem to be innervated by excitatory cholinergic

nerves. During the electrical field stimulation of the muscularis mucosae, noradrenaline increased resting tone, but inhibited the amplitude of the electrically-induced cholinergic contraction. The increase in resting tone was abolished by the pretreatment with phentolamine, while the inhibitory effect of neurogenic contraction was antagonized with a combination of yohimbine and propranolol (Christensen and Percy, 1984). These observations indicate that the dog esophageal muscularis mucosae is regulated by adrenergic nerves through postjunctional excitatory α_1- and inhibitory β-adrenoceptors or through prejunctional inhibitory α_2-adrenoceptors.

Responsiveness to drugs

Cholinomimetic drugs can evoke a sustained contraction of the esophageal muscularis mucosae through the activation of muscarinic M_3 receptors in all animal species (Kamikawa *et al.*, 1985b; Eglen and Whiting, 1988; Barocelli *et al.*, 1990; Watson *et al.*, 1995). In contrast to external smooth muscles of the gut, the contraction of the muscularis mucosae is accompanied with a weak membrane hyperpolarization, and is resistant to the voltage-gated, L-type calcium channel antagonists such as verapamil or nicardipine (Kamikawa *et al.*, 1985b; Uchida *et al.*, 1998c; Triggle, 2007). These indicate that the response is mediated by the voltage-independent, receptor-operated and store-operated calcium entry (Elliott, 2001; McFadzean and Gibson, 2002; Wray *et al.*, 2005; Thorneloe and Nelson, 2005). The store-operated calcium entry might be a subtype of transient receptor potential families (Pedersen *et al.*, 2005; Dietrich *et al.*, 2006). Exogenously applied noradrenaline and adrenaline produced a contraction of the guinea-pig muscularis mucosae with resting tone via α_1-adrenoceptors, but inhibited the sustained contraction induced by carbachol or high potassium via β_1-adrenoceptors (Kamikawa *et al.*, 1982; Uchida, 1983; Uchida *et al.*, 1983; Kamikawa and Shimo, 1987; Horinouchi *et al.*, 2003). The inhibitory response to catecholamines of the rat esophageal muscularis mucosae was mediated by the stimulation of β_3-adrenoceptors (De Boer *et al.*, 1993; De Boer *et al.*, 1995; Oostendorp *et al.*, 2004). A weak contraction mediated by α_1-adrenoceptors is thought to be an indirect action via the production of endogenous prostaglandins (PGs) from the muscularis mucosae which is enhanced in the incubation time-dependent manner (Uchida *et al.*, 1983; Uchida *et al.*, 1991). The inhibitory response to catecholamines is partly coupled with the adenylate cyclase-cyclic AMP pathway, and is further mediated by the opening of the large conductance, Ca^{2+}- activated K^+ channels and by the activation of Na^+, K^+-ATPase, since the response was partially inhibited by the pretreatment with iberiotoxin, charybdotoxin or ouabain, but not with apamin (Kamikawa and Shimo, 1987; Tanaka *et al.*, 2004; Uchida, unpublished observations). Cyclic AMP-dependent relaxants such as dibutyryl cyclic AMP, forskolin, papaverine and aminophylline equally inhibited both carbachol- and high potassium-induced tone of the muscularis mucosae. Also, trifluoperazine and quinacrine produced almost equipotent relaxation in both contractile states. The relaxant response to dibutyryl cyclic AMP was partially inhibited by the pretreatment with ouabain, but not with iberyotoxin or charybdotoxin, indicating the involvement of Na^+, K^+-ATPase, but not of Ca^{2+}-activated K^+channels (Uchida, unpublished observations). In contrast, cyclic GMP-dependent relaxants such as nitroprusside or dibutyryl cyclic GMP produced neither contraction nor relaxation of

the muscularis mucosae (Kamikawa and Shimo, 1987). These indicate that relaxation response of the muscularis mucosae mediates by the activation of adenylate cyclase-cyclic AMP pathway, but not by the guanylate cyclase-cyclic GMP pathway (Horowitz *et al.*, 1996; Beech, 1997). Cholinergic neurotransmission in the muscularis mucosae from the guinea-pig, cat, dog and opossum esophagus was inhibited by catecholamines via the stimulation of prejunctional α_2-adrenoceptors and postjunctional β_1-adrenoceptors (Kamikawa *et al.*, 1982; Christensen and Percy, 1984). Also, morphine and opioid peptides inhibited the cholinergic neurotransmission via the activation of prejunctional κ-opioid receptors but lower concentrations of serotonin enhanced that by the activation of prejunctional 5-HT_3 receptors (Kamikawa and Shimo, 1982, Kamikawa and Shimo,1983a; Kamikawa and Shimo, 1983b; Karim *et al.*, 1996). Higher concentrations of serotonin produced a transient contraction of the muscularis mucosae which was abolished by the pretreatment with tetrodotoxin or atropine, indicating an indirect action via the stimulation of intramural cholinergic nerves (Bartlet, 1968b; Kamikawa and Shimo, 1983a). In the rat esophageal muscularis mucosae, however, serotonin did not produce a contraction but relaxed the cholinergically-induced tone via the stimulation of postjunctional 5-HT_4 receptor which was coupled to the adenylate cyclase-cyclic AMP pathway (Baxter *et al.*, 1991; Moummi *et al.*, 1992; Ford *et al.*, 1992; Ohia *et al.*, 1992; Yang *et al.*, 1993; Leung *et al.*, 1996; Goldhill *et al.*, 1997). Adenosine and related purine nucleotides also enhanced cholinergic neurotransmission by the postjunctional mechanism, since the purine nucleotides produced a contraction of the muscularis mucosae via the production of endogenous PGs, probably PGE_2 (Kamikawa *et al.*, 1977; Kamikawa and Shimo 1982). Muscularis mucosae is a major source for PGs production in the digestive tracts (Lawson and Powell, 1987). In contrast to external smooth muscles, the muscularis mucosae responded to contraction by the application of arachidonic acid and its metabolites, where leukotriene C_4 and D_4 were the most potent, followed by PGE_2, and the least by $PGF_{2\alpha}$ and PGI_2 (Kamikawa and Shimo, 1979b; Kamikawa *et al.*, 1985a). We have also demonstrated the homogeneous populations of excitatory histamine H_1-receptors in the muscularis mucosae of the guinea-pig esophagus (Fujinuma *et al.*, 1985). The H_1-receptors are partly linked with the stimulation of endogenous PG biosynthesis or of intramural cholinergic nerves. Exogenously applied substance P and related tachykinins can produce a contraction of the muscularis mucosae via the stimulation of NK_2-receptors (Kamikawa and Shimo, 1984; Daniel *et al.*, 1989; Astolfi *et al.*, 1993; Holzer and Holzer-Petsche, 1997; Kerr *et al.*, 1997; Kerr *et al.*, 2000). Esophageal muscularis mucosae had two types of endothelin receptors, ET_A- and ET_B-receptors whose were linked with the phospholipase C-protein kinase C pathway (Eglen *et al.*, 1989; Uchida *et al.*, 1998a; Uchida *et al.*, 1998b; Huang, 2002). These receptors mediate tonic contractions predominantly by opening receptor-operated (store-operated) Ca^{2+} channels and partly by opening T-type Ca^{2+} channels, and mediate rhythmic motility by opening L-type Ca^{2+} channels. Neurotensin did not any direct response of the muscularis mucosae, but produced an indirect contraction by stimulating cholinergic nerves (Katsoulis and Conlon, 1988). In the rat esophageal muscularis mucosae, potassium channel openers could produce a relaxation through an increase in K^+ permeability that is coupled to potential-operated Ca^{2+} influx (Akbarali *et al.*, 1988a; Akbarali *et al.*, 1988b). Akbarali and Giles (1993) first reported electrophysiological characteristics of the rabbit esophageal muscularis mucosae using a whole-

cell gigaseal technique, where only one type of Ca^{2+} current could be identified. Since pharmacological responsiveness of the esophageal muscularis mucosae thus had a variety of species-difference, their exact subcellular mechanisms have not yet been clarified.

Clinical implications

Kuwano *et al.* (1989) first reported that the lack of muscularis mucosae existed in patients with spontaneous ruputure of the esophagus. They suggest that the lack of muscularis mucosae may be linked to Boerhaave's syndrome. The muscularis mucosae may well act as bumper against increased intraluminal pressure of the esophagus. Esophageal achalasia is characterized by abnormalities of peristalsis of the esophageal body and of the lower esophageal sphincter to relax in response to swallowing (Cohen, 1979). In achalasia, lesions have been found in vagal nerves and myenteric plexus where cholinergic, VIP-ergic and dopaminergic neurotransmissions to smooth muscles were hyporesponsive (Smith, 1970; Holloway *et al.*, 1986; Sigala *et al.*, 1995). These abnormalities might inhibit motor activity of the muscularis mucosae. Similar peristaltic dysfunction is also found in peptic esophagitis where acid clearance is lowered (Kahrilas *et al.*, 1986). We have demonstrated that large numbers of connective tissue mast cells were found in the lamina propria and muscularis mucosae, but not in stratified squamous epithelium of mammalian esophagus (Fujinuma *et al.*, 1986). Furthermore, anaphylactic challenge with ovalbumin or treatment with compound 48/80, a mast cell stimulant, of the guinea-pig esophageal muscularis mucosae caused a contraction which was mediated by the lipoxygenase products of arachidonic acid (Fujinuma *et al.*, 1987; Fujinuma, 1988). These suggest that esophageal muscularis mucosae is a major site of allergic inflammation.

Gastric muscularis mucosae

Autonomic innervations

Gastric muscularis mucosae is composed of two types of smooth muscles, outer longitudinal and inner circular. Some smooth muscle fibers pass up between glands to be attached to epithelial basement membrane (Freeman and Bracegirdle, 1967). Nerve fibers containing substance P-, VIP- , neuropeptide Y-, and enkephalin-like immunoreactivity are present in the gastric muscularis mucosae and in the adjacent submucous plexus (Holzer *et al.*, 1981; Keast *et al.*, 1985; Holzer and Holzer-Petsche, 1997).

(1) Human: The muscularis mucosae isolated from different directions of the human stomach spontaneously developed resting tone and motor activity whose were varied from preparation to preparation (Walder, 1953). Nicotine produced both contractile and relaxant responses of the muscularis mucosae which were also different from preparation to preparation. Since both responses were blocked by the pretreatment with hexamethonium or with atropine and ergotoxin, it is suggested that nicotine can activate intramural excitatory cholinergic nerves and inhibitory adrenergic nerves. We have investigated the neurogenic response evoked by electrical field stimulation of the human gastric muscularis mucosae (Kamikawa *et al.*, 2005; Uchida *et al.*, 2005). Electrical field stimulation of the isolated muscularis mucosae evoked a

relaxation in a frequency-dependent manner, which was abolished by the pretreatment with tetrodotoxin or L-nitroarginine methyl ester (L-NAME). The electrically-induced relaxation was reversed to a contraction in the presence of L-NAME which was slightly inhibited by atropine. In some preparations, electrical stimulation at low frequencies induced a relaxation which was blocked by propranolol. Our observations suggest that the human gastric muscularis mucosae is innervated mainly by inhibitory nitrergic nerves and partly by excitatory cholinergic nerves and inhibitory adrenergic nerves.

(2) Guinea-Pig: The muscularis mucosae isolated from fundic area of the guinea-pig stomach developed resting tone and spontaneous motor activity. Exogenously applied carbachol produced a biphasic contraction consisting of an initial transient contraction followed by a sustained contraction. The pretreatment with tetrodotoxin did not inhibit the carbachol-induced biphasic contraction, but rather augmented the amplitude of the initial contraction, indicating the involvement of intramural inhibitory nerves (Sukigara, 1991).

(3) Rat: The inner layer of the lamina muscularis mucosae of the rat stomach sends strands of smooth muscle between the gastric glands. An oscillating gland luminal pressure probably results in intermittent emptying of the glandular contents. The oscillation may be generated by rhythmic contractions of the muscularis mucosae or the connected muscle strands. In the rat stomach, VIP, a putative inhibitory neurotransmitter in the gastrointestinal tract, significantly decreases the glandular pressure, and the amplitude of the pressure oscillations is also reduced by VIP. VIP-immunoreactive nerve terminals are observed around glands and pits in gastric mucosa, and VIP-reactive fibers are numerously found in the muscularis mucosae (Schultzberg *et al.*, 1980; Synnerstad *et al.*, 1998). VIP-containing neurons may play a physiological role for regulation of gland luminal pressure via modulation of the tone in the muscularis mucosae and the connected muscle strands.

(4) Rabbit: The muscularis mucosae isolated from fundic or antral end of the rabbit gastric corpus developed resting tone and spontaneous motor activity in some preparations. Electrical field stimulation of the muscularis mucosae evoked a biphasic response, consisting of an initial contraction followed by a sustained relaxation. Although both components were abolished by the pretreatment with tetrodotoxin, neither initial contraction nor following relaxation were inhibited with atropine, propranolol or L-NAME (Percy and Warren, 1994; Percy *et al.*, 1999). The rabbit gastric muscularis mucosae is presumably innervated by excitatory non-cholinergic nerves and inhibitory non-adrenergic and non-nitrergic nerves, but each neurotransmitter is still unclear.

(5) Dog: The muscularis mucosae isolated from the dog antral stomach developed resting tone and spontaneous motor activity. Electrical field stimulation evoked a relaxation and inhibited spontaneous motor activity. The inhibitory response was abolished by the pretreatment with tetrodotoxin, but not with atropine, phentolamine, propranolol or methysergide (Angel *et al.*, 1982; Angel *et al.*, 1983). These findings indicate that the dog gastric muscularis mucosae does not seem to be mediated by cholinergic, adrenergic or serotonergic nerves. Since VIP produced a relaxation of the antral muscularis mucosae and in the presence of VIP antiserum the electrically-induced inhibitory response was abolished, VIP may function as a non-adrenergic and non-cholinergic inhibitory neurotransmitter in the lamina muscularis mucosae of the dog gastric antrum (Angel *et al.*, 1983; Morgan *et al.*, 1985).

Responsiveness to drugs

There are few reports on pharmacological experiments using the gastric muscularis mucosae. Human gastric muscularis mucosae showed contraction to acetylcholine, but is insensitive to histamine (Walder, 1953). In contrast to human, dog gastric muscularis mucosae possessed both contractile H_1 and relaxant H_2 receptors (Muller *et al.*, 1993). Rabbit gastric muscularis mucosae contracted to acetylcholine, ATP and histamine, but relaxed to VIP (Percy *et al.*, 1999). Adenosine, cholecystokinin, gastrin, secretin and somatostatin were without effect (Muller *et al.*, 1994). Rat gastric muscularis mucosae contracted to acetylcholine and adrenaline, but not to 5-HT or histamine (Horn and Zweifach, 1963). The responsiveness to drugs showed a greater regional difference in gastric body, where fundic but not antral muscularis mucosae contracted to bombesin, PGE_2 and $PGF_{2\alpha}$ (Percy *et al.*, 1999).

Clinical implications

Since the neuronally-mediated relaxation of the gastric muscularis mucosae seems to facilitate acid release by opening the gastric glands (Synnerstad *et al.*, 1998), dysfunction of VIP-ergic nerve-muscularis mucosae transmission may contribute to peptic ulcer (Percy *et al.*, 1999).

Intestinal muscularis mucosae

Autonomic innervations

The muscularis mucosae distributed in small and large intestine consisted from a very thin layer of outer longitudinal and inner circular smooth muscles (Freeman and Bracegirdle, 1967). Earlier physiological studies by King and his colleagues (King and Arnold, 1922; King *et al.*, 1922; King and Church, 1923; King and Robinson, 1945; King *et al.*, 1947) had demonstrated that the muscularis mucosae of the dog small intestine was innervated by both cholinergic and adrenergic excitatory nerves. There is no evidence for the presence of an inhibitory innervation. Nerve fibers containing substance P-, VIP- , NPY-, somatostatin- and enkephalin-like immunoreactivity are present in the intestinal muscularis mucosae and in the adjacent submucous plexus of all animal species including human (Holzer *et al.*, 1981; Keast *et al.*, 1985). Recent evidence indicates that the muscularis mucosae of the large intestine is innervated abundantly by excitatory tachykininergic nerves and inhibitory VIP-egic and CGRP-ergic nerves, sparsely by excitatory cholinergic nerves, but not by adrenergic nerves (Keast *et al.*, 1985; Holzer and Holzer-Petsche, 1997). Nerve fibers containing somatostatin-, NPY- and enkephalin-immunoreactivity are also distributed in the colonic muscularis mucosae.

(1) Human: We recently observed that electrical field stimulation of the muscularis mucosae isolated from the human distal colon evoked a rapid relaxation in a frequency-dependent manner (Kamikawa *et al.*, 2005; Uchida *et al.*, 2005). The electrically-evoked rapid relaxation was abolished by the pretreatment with tetrodotoxin or L-NAME. Electrical stimulation of the L-NAME-treated muscularis mucosae produced a fast contraction which was abolished by the atropine pretreatment. In the presence of both L-NAME and atropine, electrical stimulation of the muscularis mucosae produced a slow relaxation which was blocked by the further treatment with propranolol. These findings indicate that the muscularis mucosae

of the human distal colon is innervated mostly by inhibitory nitrergic nerves and slightly by excitatory cholinergic and inhibitory adrenergic nerves.

(2) Guinea-Pig: The longitudinal muscularis mucosae isolated from the guinea-pig colon usually showed spontaneous rhythmic activity (Ishikawa and Ozaki, 1997; Kamikawa *et al.*, 2002). Electrical field stimulation of the colonic muscularis mucosae evoked a tetrodotoxin-sensitive biphasic contraction. The pretreatment with atropine abolished the first contraction, but not the second contraction. These suggest that the muscularis mucosae of the guinea-pig proximal colon receives functional innervation by excitatory cholinergic and non-cholinergic nerves (Ishikawa and Ono, 1992). Although various neuropeptides were found in the enteric nervous system of the guinea-pig colon, substance P or related tachykinins might be a most probable candidate for the non-cholinergic neurotransmitter (Ishikawa and Ozaki, 1997; Kamikawa *et al.*, 2002).

(3) Rabbit: The muscularis mucosae isolated from the rabbit colon showed variable spontaneous rhythmic activities (Gallacher *et al.*, 1973; Percy *et al.*, 1992). Electrical field stimulation of the muscularis mucosae from the proximal colon produced a contraction which was abolished by the pretreatment with tetrodotoxin or atropine. Electrical stimulation of the muscularis mucosae from the distal colon produced a biphasic response consisting of an initial contraction followed by a relaxation. The initial contraction was also blocked by the tetrodotoxin or atropine treatment, but the subsequent relaxation was unaffected by the propranolol or L-N^{ω}-nitro-arginine treatment (Gallacher *et al.*, 1973; Percy *et al.*, 1992). These observations suggest that the muscularis mucosae of the rabbit proximal colon is solely innervated by excitatory cholinergic nerves but that of the distal colon is innervated by both excitatory cholinergic nerves and inhibitory non-adrenergic and non-nitrergic nerves.

(4) Opossum: The muscularis mucosae isolated from the opossum distal colon spontaneously developed resting tone and rhythmic activity. Electrical field stimulation of the colonic muscularis mucosae produced a biphasic response, consisting of an initial contraction followed by a relaxation. The biphasic response was abolished by the pretreatment with tetrodotoxin. The initial contraction was further abolished by the atropine treatment, but the subsequent relaxation was unaffected by the phentolamine or propranolol treatment (Percy and Christensen, 1986). These findings indicate that the colonic muscularis mucosae receives both excitatory cholinergic nerves and inhibitory non-adrenergic and non-cholinergic nerves.

(5) Cat: A ganglionic stimulant, nicotine produced only a relaxation of the cat colonic muscularis mucosae which was abolished by the pretreatment with tetrodotoxin or hexamethonium. The pretreatment with propranolol, guanethidine, bretylium or reserpine partially blocked the nicotine-induced relaxation. Furthermore, high potassium produced a biphasic response of the muscularis mucosae, consisting of an initial transient relaxation followed by a sustained contraction. The initial relaxation, but not the sustained contraction, was abolished by the tetrodotoxin pretreatment, and partly inhibited by the propranolol or guanethidine pretreatment (Onori *et al.*, 1971). These indicate that the cat colonic muscularis mucosae is innervated by inhibitory adrenergic and non-adrenergic nerves.

(6) Dog: The muscularis mucosae isolated from the dog proximal colon spontaneously developed resting tone and rhythmic activity. Electrical field stimulation of the muscularis

mucosae produced a biphasic response consisting of an initial contraction followed by a relaxation. The biphasic response was completely blocked by the pretreatment with tetrodotoxin, but not with atropine, phentolamine or propranolol. The contractile and relaxant components to electrical stimulation were abolished by the substance P-antiserum and VIP-antiserum pretreatment, respectively (Angel *et al.*, 1982; Angel *et al.*, 1984). These observations suggest that the dog colonic muscularis mucosae is innervated by excitatory tachykininergic nerves and inhibitory VIP-ergic nerves.

Responsiveness to drugs

We have shown that contractile responsiveness of the colonic muscularis mucosae to drugs is different from species to species (Kamikawa *et al.*, 2002). In the human colon, neurokinin A was the most potent, followed by carbachol and $PGF_{2\alpha}$, but histamine, serotonin and bradykinin were negligible. In contrast, bradykinin was the most potent but $PGF_{2\alpha}$ was negligible in the rat colon. Furthermore, the muscularis mucosae of the rabbit colon exhibited distinct behavior and pharmacologic properties from proximal to distal colon (Percy *et al.*, 1992). Using the whole cell patch-clamp technique, Hatakeyama *et al.* (1996) have demonstrated that tyrosine kinase modulates the entry of Ca^{2+} through both L-type calcium channels and store-operated calcium channels. In contrast to external longitudinal smooth muscles, adenosine and related purine compounds caused a contraction of the intestinal muscularis mucosae via the activation of A_1- or A_{2b}- adenosine receptors and P_{2X}- or P_{2Y}-purinoceptors, respectively (Hourani *et al.*, 1993; Reeves *et al.*, 1995; Brownhill *et al.*, 1996; Brownhill *et al.*, 1997; Johnson *et al.*, 1996; Nicholls *et al.*, 1996; Nicholls and Hourani, 1997; Hourani *et al.*, 1998; Peachey *et al.*, 1999). A part of the former contraction was mediated by the stimulation of cyclooxygenase pathway.

Clinical implications

Since intestinal muscularis mucosae is invaded into the villi, inflammatory and malabsorptive diseases may accompany with dysfunction of the muscularis mucosae (Barbara *et al.,* 2004). We have demonstrated that large numbers of mucosal mast cells were found in the lamina propria and muscularis mucosae, but scarcely in the epithelium and external muscles of the mammalian ileum (Fujinuma *et al.*, 1986). Furthermore, Woodbury *et al.* (1984) had reported that the number of mucosal mast cells per villus crypt in the rat duodenum increased with parasitosis. The mucosal mast cells may therefore function as an expulsion system of intestinal nematode infections. In recent, O'Hara *et al.* (2004) presented evidence that enteroendocrine cells and serotonin availability in intestinal mucosa were altered in experimental ileitis. This indicates that pathological changes in the transduction pathway between mucosal endocrine cells and primary afferent nerve terminals may lead to decreased motility and secretion in irritable bowel diseases. A number of studies by Percy and his colleagues (Percy and Christensen, 1986; Percy *et al.*, 1986; Percy *et al.*, 1992; Percy *et al.*, 1993a; Percy *et al.*, 1993b; Percy and Warren, 1994; Percy *et al.*, 1997; Percy *et al.*, 1998; Percy *et al.*, 1999; Percy *et al.*, 2001) have revealed that contractility of the muscularis mucosae closely linked with mucosal secretory function and its abnormal motility was concerned in inflammatory bowel diseases. As this physiological transduction mechanism, muscularis mucosae motor

activity is translocated into mucosal secretion via the contraction-related PG synthesis and stimulation of non-cholinergic secretomotor neuron (Lawson and Powell, 1987; Percy *et al.*, 2003). Pathophysiological changes in the muscularis mucosae motor activity might involve the altered mucosal barrier function for bacterial adherence and proliferation (Percy *et al.*, 1998). Evidence that intestinal muscularis mucosae largely responded to $PGF_{2\alpha}$, LTC_4 and LTD_4 but not to histamine or serotonin suggests that in inflammatory bowel diseases elevated arachidonic acid metabolites may cause hyperirritability of the muscularis mucosae leading to diarrhea or constipation (Sharon and Stenson, 1984; Hawkey and Rampton, 1985; Lauritsen *et al.*, 1988; Percy *et al.*, 1993a; Percy *et al.*, 1993b; Kamikawa *et al.*, 2002). In the experimental rat model for colitis, pathological changes such as inflammatory cell infiltration, edema, hemorrhage and metaplasia were observed in the muscularis mucosae of the large intestine (Yotsuya *et al.*, 2001). As a new therapeutic target for inflammatory bowel diseases such as diarrhea, constipation, irritable bowel syndrome and Crohn's disease (Kirsner, 2000; Van Montfrans *et al.*, 2002; Lembo and Camilleri, 2003; Mertz, 2003), much attention should be attracted to the motor regulation of the intestinal muscularis mucosae.

Conclusion

Autonomic innervations or physiological and pharmacological responsiveness of the muscularis mucosae in the digestive tract had been different from the external longitudinal and circular smooth muscle layers. In addition, the muscularis mucosae had different profiles for innervation and responsiveness in a species- and regional-specific manner. Culture of the muscularis mucosae is still unsuccessful. Since electrophysiological studies using the muscularis mucosae are not extensively carried out, exact characteristics of ion channels located on the muscularis mucosae are still unknown. Thus, the physiological and pharmacological studies of the muscularis mucosae have been virtually neglected to date. Because of its strategic location below the absorptive and secretory apparatus of the bowel, abnormalities in its behavior may contribute to some bowel diseases. For example, in ulcerative colitis and ileitis, the striking pathological findings are hypertrophy and contraction of the muscularis mucosae. These changes undoubtedly lead to changes in motility of the muscularis mucosae. Pathological conditions involving the muscularis mucosae include achalasia, peptic ulcer, cancer invasion, vomiting, constipation, diarrhea, irritable bowel syndrome and Crohn's disease. As future therapeutic targets in these diseases, much attention should be attracted to the regulation of motor activity of the human muscularis mucosae.

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