Functional Coordination of Motor Activity in Colonic Smooth Muscles in Rat Experimental Model

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Summary
Spontaneous and electrically-elicited motor activity was recorded by triple organ bath in rat segment-model preparation as display of excitation of local nerve networks and ascending or descending reflex pathways underlying contractile potency and functional coordination of colonic longitudinal and circular muscles. Spontaneous high-amplitude contractions, but not relaxations, appeared synchronously in both muscles. Electrical field stimulation applied to proximal or distal part of segments elicited both tetrodotoxin (0.1 µM)-sensitive local motor responses of the stimulated part and ascending or descending motor responses of the contralateral, nonstimulated part of the preparations. Contractions characterized the local response of longitudinal muscle. The circular muscle responded with relaxation followed by contraction. Synchronous ascending contractions and descending contraction of the longitudinal muscle and relaxation followed by contraction of the circular muscle were observed when the middle part of segments was stimulated, thus indicating that locally-induced nerve excitation propagated via intrinsic ascending or descending nerve pathways that could be synchronously coactivated by one and the same stimulus. The ascending motor responses were more pronounced and the motor responses of longitudinal muscle were expressed more than those of circular muscle suggesting an essential role of ascending reflex pathways and longitudinal muscle in the coordinated motor activity of colon.

Key words
Local networks • Motor reflexes • Colon

Introduction
Because of physiological significance of colon as reservoir or conduit organ and healthy impact as target of tumors the motility reflexes of colonic region are subject of experimental and clinical studies. Colo-rectal stimulatory (Van der Veek et al. 2004), recto-colonic inhibitory (Bampton et al. 2002, Law et al. 2002) as well as colo-anal (Malcolm and Camilleri 2000) reflex pathways have been demonstrated. Recent observations turn attention on the coordination of motor activity between colonic and recto-anal muscles (Brading et al. 2008) and on the role of stretch and elongation on the motility of colon. The distension could generate peristaltic waves in guinea pig distal colon (Smith et al. 2003). The transit in the guinea pig distal colon (Dickson et al. 2007) and polarized reflexes in murine colon can be triggered by elongation in the longitudinal axis (Dickson et al. 2008).

Despite that knowledge on the colon motility has advanced considerable the nerve circuitry mediating motor activity of longitudinal and circular muscles is not fully understood. Enteric pathways form overlapping networks which can be regarded as local modules. The spatiotemporal coordination of modules is determining factor for the generation of motor patterns (Costa et al. 2000). The role of local nerve networks underlying contractile and/or relaxant activity of longitudinal and circular muscle in different colonic region requires further elucidation. The functional coordination of colonic muscles is a matter of debate. Synchronous
movements of the longitudinal and circular muscle were demonstrated in the guinea pig distal colon (Smith and Robertson 1998) while reciprocal activity of both muscles was observed in rat colon (Grider 2003).

In the present study, we have reexamined nerve-mediated motility of colon using a segment isolated from rat as an experimental model. In particular we were interested in contractile potency and functional coordination of longitudinal and circular muscle underlying by local and ascending and descending reflex pathways. The nerve structures were excited by electrical field stimulation. Partitioned organ bath method allowing the application of electrical stimulation to the proximal, middle or distal part of the colonic segment and simultaneous registration of the motor responses of longitudinal and circular muscles belonging to the proximal or distal region of the preparation was used.

Methods

The experiments were carried out in the Laboratory of Peripheral synapses of the Institute of Neurobiology, Bulgarian Academy of Sciences in accordance of Ethics Committee of the Institute of Neurobiology.

Animals

Male rats weighing 250-280 g were sacrificed. The animals were starved overnight, stunned by a blow on the neck and decapitated. The abdominal cavity was opened and the distal part of large intestine was removed. Segment of colon with intact nerve plexuses-smooth muscle layers, 50-52 mm in length was isolated as experimental model-preparation and mounted horizontally in triple organ bath.

Triple organ bath

The organ bath was divided into three compartments by plastic partitions with slits filled by paraffin “diaphragms”, thus consisting of oral, middle (length: 10 mm) and anal compartments. The segment was threaded through a 2 mm diameter hole in each paraffin diaphragm with the proximal and distal ends of the preparation lying in the oral and the anal compartments, respectively. Inert silicone grease was then applied around the gut circumference in the paraffin diaphragms to seal any possible contact between solutions in the compartments (Ivancheva and Radomirov 2001).

The proximal and distal parts of the colonic segment-preparations, each with a length of 20-21 mm, were mounted over plastic rods. The outer part of each rod (length, 10 mm) was bent up at an angle of 45 degrees from the horizontally situated inner part. The proximal and distal parts of the segments were secured by thread to the rods at the bend. Thus, interference of the movements in the longitudinal or circular axis at the points of recordings was minimized. The ends of the segments were tied by a thread to strain gauges to measure movements in the longitudinal axis. The motor activity of the circular muscle from proximal or distal parts of the segments was measured between two opposite sites of the ring circumference. The longitudinal and circular muscles were stretched at an initial load equivalent to 10 mN. The motor activity of both muscles belonging to proximal and distal parts of the colonic segments was simultaneously registered (Brading et al. 2008).

Electrical stimulation

Electrical field stimulation (EFS) was used to excite the nerve structures of the isolated segments (Paton and Vizi 1969). EFS (0.8 ms, 40 V, 2-5-10 Hz, 20 s) was applied by means of two platinum electrodes (0.45 mm thick) placed diametrically opposed along the sides of the compartments at a separation of 14 mm at interval not less than 5 min (Ivancheva and Radomirov 2001). The responses to 5 Hz-stimulation resembled by pattern and amplitude to the spontaneous high-amplitude contractions and considered as suitable for comparison of responsiveness of both muscles.

The application of EFS either in the oral or in anal compartment of the bath elicited motor responses of the longitudinal and circular muscles from the stimulated part of colonic segments. These responses were considered as ‘local’ responses due to excitation of local nerve networks lying in the field of stimulation. At the same time motor responses of the contralateral, non-stimulated part of the segments at a distance of 20 mm were observed. These responses were considered as ‘ascending or descending’ responses due to propagation of excitation via orally or anally directed reflex pathways. The stimulation of the middle part of segments elicited responses of both muscles belonging to proximal and distal part of the preparations at a distance of 10 mm. To evaluate the declination of excitation the ascending responses were compared to the local responses of proximal part of the colonic segments. The descending
responses were compared to the local responses of the distal part.

The mechanographic techniques were completed by strain gauges and amplifiers (Microtechna, Prague, Czech Republic), stimulators (Experimetria, Budapest, Hungary), 6-channels line recording devices (Watanabe, Tokyo, Japan) and recorders TZ 4620 (Laboratorní pristroje, Prague, Czech Republic).

**Solution and drug**

The composition of the Krebs bath solution was (mM): NaCl 120, KCl 5.9, NaHCO₃ 15.4, NaH₂PO₄ 1.2, MgCl₂ 1.2, CaCl₂ 2.5 and glucose 11.5. The solution was continuously aerated by 95 % O₂ and 5 % CO₂ (pH 7.2) at 36.5 °C. Tetrodotoxin (TTX, Sankyo, Zürich, Switzerland) was used to verify the nerve-mediated nature of the electrically-elicited responses. The stock solution of TTX was stored at –18 °C.

**Statistics**

Data are presented as mean values ± S.E.M. Statistical significance was assessed by Student’s ‘t’ test for paired and unpaired data at p<0.05 using computer programs.

**Results**

**Spontaneous motor activity**

High-amplitude irregular contractions characterized the spontaneous motor activity of longitudinal and circular muscles of colonic segment-preparations. The contractions could be considered by visual inspection as moving motor complex propagating from the proximal to distal part of the segments. The amplitudes of contractions of the longitudinal muscle were significantly higher than those of the circular muscle (proximal part, longitudinal muscle, 10.4±2.3 mN and circular muscle, 5.0±0.9 mN, n=52, p<0.05; distal part, longitudinal muscle, 9.7±2.5 mN and circular muscle, 5.3±1.1 mN, n=48, p<0.05) (Fig. 1).

**Electrically-elicited local motor responses**

The local motor responses of the longitudinal muscle from the proximal and distal parts of colonic segments were contractions. The responses of the circular muscle consisted of initial relaxation followed by contraction. The responses of the muscles belonging to both parts of colonic segments were similar by pattern (Fig. 2 A, D). There were no differences between the amplitudes in the local motor responses of longitudinal muscle recorded either from proximal or distal parts of segments (Fig. 2 B, E). The amplitudes of the relaxation and the contraction in the local responses of circular muscle did not considerable differ in either part of the colonic preparations (Fig. 2 C, F).

The contractions in the local responses of the circular muscle induced by 5-Hz EFS were significantly less pronounced as compared to local contractile responses of longitudinal muscle (proximal part, longitudinal muscle, 17.5±2.1 mN and circular muscle, 9.8±1.4 mN, n=12, p<0.05; distal part, longitudinal muscle, 16.9±1.9 mN and circular muscle, 9.7±1.2 mN, n=12, p<0.05, respectively).

**Electrically-elicited ascending motor responses**

The application of EFS to the middle part of colonic segment-preparations elicited ascending motor responses of the longitudinal and circular muscle of the proximal part of segments (Fig. 3 A) and descending motor responses in both muscles of the distal part of segments (Fig. 4 A) at a distance of 10 mm. The application of EFS to the distal part of colonic segments induced ascending motor responses in the longitudinal and circular muscles of proximal part of segments at a distance of 20 mm (Fig. 3 D).
The ascending motor responses were contractions that synchronously appeared in both muscles and did not differ by pattern (Fig. 3 A, D). The amplitudes of the contractile responses of the longitudinal muscle (Fig. 3 B, E) were considerably higher than the amplitudes of contractions of the circular muscle (Fig. 3 C, F). A decrease of the ascending responses with the lengthening of the distance from the application of EFS was observed in both longitudinal (Fig. 3 B, E) and circular (Fig. 3 C, F) muscles. The amplitudes of the longitudinal and circular muscles to 5-Hz stimulation applied at distance of 10 mm or 20 mm significantly differed (at a distance of 10 mm, longitudinal muscle, \(13.7\pm1.2\) mN and circular muscle, \(4.0\pm0.6\) mN, \(n=12\), \(p<0.05\); at a distance of 20 mm, longitudinal muscle, \(8.7\pm0.8\) mN and circular muscle, \(2.8\pm0.5\) mN, \(n=10\), \(p<0.05\)).

The ascending contractile responses were significantly lower by amplitude as compared to the corresponding local contractile responses of the longitudinal (Fig. 2 B, Fig. 3 B) or the circular (Fig. 2 C, Fig. 3 C) muscles.
The ascending motor responses were significantly more pronounced in the longitudinal muscle. The ascending contractile responses of the longitudinal muscle elicited by EFS at a distance of 20 mm reached 49.9±6.3 % (n=10, p<0.05) of the local responses while the ascending contractions of the circular muscle were 29.0±3.3 % (n=10, p<0.05) of the contractions in the local responses (Fig. 5 A). A similar correlation between the ascending motor responses in both muscles was observed when EFS was applied at the shorter distance of 10 mm (Fig. 5 B).

Electrically-elicited descending motor responses

Simultaneous descending motor responses of the longitudinal and circular muscles belonged to the distal part of the colonic segments were registered at a distance of 10 mm (Fig. 4 A) or 20 mm (Fig. 4 D) when the middle or proximal part of the segment-preparations was electrically stimulated.

The descending motor responses of the longitudinal muscle were contractions while the descending responses of the circular muscle comprised by a relaxation followed by contraction (Fig. 4 A, D). The descending responses of both muscles resembled by pattern the local responses of distal part of colonic segments although were less pronounced (Fig. 2 D, Fig. 4 A, D). The descending contractile responses of the longitudinal muscle were higher in amplitude than the contractions in the descending responses of the circular muscle and to 5-Hz stimulation were significantly different (at a distance of 10 mm, longitudinal muscle, 6.5±0.9 mN and circular muscle, 3.9±0.6 mN, n=12, p<0.05; at a distance of 20 mm, longitudinal muscle, 3.6±0.6 mN and circular muscle, 2.0±0.4 mN, n=12, p<0.05).

The descending contractile responses of longitudinal muscle were less pronounced than the ascending contractile responses as compared to the respective local responses. There were no differences between the ascending and descending contractions of the circular muscle when they were compared to the local responses. The descending motor response in both muscles decreased when the distance from the application of EFS was increased (Fig. 5 A, B). The descending contraction of longitudinal muscle as well as the descending relaxation and contraction of circular muscle in response to EFS applied at a distance of 20 mm were 21.5±3.8 % (n=12, p<0.05) and 32.0±3.4 % (n=12, p<0.05) and 20.7±3.1 % (n=12, p<0.05) of the respective local responses of the distal part of colonic segments.

Effect of TTX

The addition of TTX (0.1 μM) for 10 min (n=4) to proximal or distal part of colonic segments abolished the local motor responses of both muscles. When TTX was added to the middle part of segments EFS did not induce ascending or descending reflex motor responses (data not shown).

Discussion

The present electrically-elicited motor responses of longitudinal and circular muscles of segment-preparations isolated from rat colon were neurogenic by nature since they were prevented by TTX, a blocker of neuronal conductance. The triple organ bath allowed simultaneous recording of motor activity from the proximal and distal parts of colonic segments and made it possible to compare the local motor responses versus the ascending or descending reflex motor responses due to activation of nerve pathways involved in colonic integrative circuitry.

Spontaneous contractility have been described in the colon of pig (Shafik et al. 2001), human (Bassotti et al. 2005) and rat (Mitsui et al. 2005). The migrating motor complex in the mouse colon (Brierley et al. 2001) and giant contractions in rat distal colon (Mitsui et al. 2005) have been attributed to the contractile activity of circular muscle. We observed high amplitude contractions that appeared synchronously in the
longitudinal and circular muscles, thus suggesting co-
activation of nerve pathways supplying both muscles. The contractions propagated from proximal to distal part of the preparations, thus showing descending excitatory pathways and confirming the data for localization of pacemaker controlling colonic muscle activity in the proximal region of colon. More recently, time-delay in the appearance of contractile activity in the distal part of rat colonic isolated segments has been estimated by the conduction velocity determined by the length of the segment between the recording points (Brading et al. 2008). A colonic pacemaker to control lower colonic and rectal movements was described in mongrel dogs (Hirabayashi et al. 2003). The giant migrating contractions of the colon can propagate to the rectum and anal sphincters (Malcolm and Camilleri 2000, Bassotti et al. 2005).

Relaxations appeared by EFS-induced excitation of local and reflex nerve structures. The effect of electrical stimulation is the consequence of propagation of action potential causing local release of excitatory and/or inhibitory neurotransmitters (Paton and Vizi 1969, Kadlec et al. 1990). The application of EFS either to proximal or to distal part of the colonic segments elicited motor responses of the stimulated part as result of excitation of local nerve structures. The increase of stimulus frequency enhanced the amplitude without changing the pattern of responses suggesting release of the same neurotransmitters. Electrical stimuli applied at higher frequencies recruit more nerve terminals into the process of neurotransmitter release (Kadlec et al. 1993). We did not observe differences between the electrically-induced contractions of longitudinal muscle recorded either from the proximal or the distal part of the colonic segments. The relaxations of the circular muscle from both parts of the preparations were similar by pattern and amplitude. These findings indicate similarity of local nerve networks in different parts of rat colon. Lack of differences in activity of longitudinal muscle along the length of colon during distension was reported in pig. There were no changes in the amplitude, frequency and conduction velocity of the slow waves recorded by electrodes placed at 3 positions (Shafik et al. 2001).

Electrical stimulation applied to the distal or to proximal part of the colonic segments induced ascending or descending motor responses in the non-stimulated contralateral part of the preparations indicating that the locally induced nerve excitation propagated via ascending or descending nerve pathways. Simultaneous ascending and descending motor responses of both muscles were observed when the middle part of the segments was stimulated, thus demonstrating that ascending and descending reflex pathways could be synchronously co-activated by the same stimulus. The ascending motor responses of both muscles were contractions. We did not register ascending relaxation of the longitudinal muscle described in flat-sheet preparation of rat colon (Grider 2003). The difference in the pattern of the ascending responses of longitudinal muscle could be attributed to the type of the model-preparations used. An initial electrically-elicited relaxation appeared in the descending motor responses of the colonic circular muscle but not in longitudinal muscle indicating that descending reflex pathways involve inhibitory neurons to supply only the circular muscle. The present finding is in accordance with the view that colon motility relies on the activation of ascending excitatory and descending inhibitory nervous pathways to circular muscle (Grider and Macklouf 1986, Smith and Robertson 1998). Recent observations turn attention on the role of circular muscle in transducing signals that underlie repetitive firing of ascending excitatory and descending inhibitory neuronal pathways (Spencer et al. 2006).

Electrophysiological studies demonstrated that excitatory or inhibitory junction potentials in mouse colonic circular muscle were dependent on the distance between the site of application of electrical stimulation and the recording site. Excitatory junction potentials could be recorded over long distances with maximum amplitude at a distance of 10 mm. At distances greater than 10 mm apart, electrical stimulation did not produce inhibitory junction potentials (Sibaev et al. 2008). The present data obtained by mechanographic techniques showed that neuromuscular communications in rat colon provided motor responses at distance at least of 20 mm.

The spontaneous contractions as well as the electrically-elicted contractile local and ascending or descending responses of the longitudinal muscle were considerably more pronounced than those of the circular muscle suggesting a predominant activity of longitudinal muscle in the colonic motility. Most probably the difference in responsibility of both muscles is due to specific organization of colonic nerve networks involving prevalence of excitatory neurons to the longitudinal muscle and inhibitory innervation supplying the circular muscle. Longitudinal muscle activity in rat colon is regulated by interneurons coupled to cholinergic and tachykinergic motor neurons releasing excitatory
neurotransmitters acetylcholine and tachykinins (Grider 2003). Enteric inhibitory motor neurons supplying the circular muscle use multiple non-adrenergic non-cholinergic mechanisms of inhibitory transmission including nitric oxide, adenosine triphosphate and peptides as vasoactive intestinal peptide acting directly on smooth muscle or indirectly via interstitial cells (Costa et al. 2000). The release of the inhibitory neurotransmitter nitric oxide to nerve stimulation in human colon is greater in circular than in longitudinal muscle (McKirdy et al. 2004).

The physiological impact of the different regions of large intestine in stool movement is a matter of debate (Shafik et al. 2006). We found that the ascending contractions of longitudinal and circular muscles in rat colonic segments were more pronounced than the descending responses. Probably, this finding could be interpreted that the propagation of excitation was expressed more in ascending than in descending reflex pathways. Our data obtained in colon are in accordance with experiments performed in recto-anal region (Radomirov et al. 2009) and small intestine (Spencer et al. 2000, Ivancheva and Radomirov 2001) showing that the contractions of longitudinal and circular muscles induced by electrical or mucosal brush stimulation applied anally to the recording point were higher as compared to the responses to orally applied activation. The ascending and descending reflex motor responses in both longitudinal and circular muscles decreased with the increase of the distance between the stimulation site and recording points which supports the view that the propagation of electrically-evoked excitation in nerve structures depends on the distance from the application of stimulation (Kadlec et al. 1990).

In summary, there were no differences between the local responses recorded either in the proximal or distal part of the colonic segments suggesting identical organization of local nerve networks in different parts of colon. Simultaneous motor responses of the longitudinal and circular muscles revealed in proximal and distal parts of the colon indicating that excitatory and inhibitory reflex pathways underlying ascending contraction in both muscles and descending relaxation in the circular muscle could be activated by the same stimulus. The more pronounced contractility of the longitudinal muscle and the higher ascending reflex-mediated contractile potency determining the propulsive capacity indicate an essential role of the longitudinal muscle in the coordinated motor activity and suggest that colon is rather a conduit than a storage organ.

Conflict of Interest
There is no conflict of interest.

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