

CHARACTERIZATION OF COLONIC ASCENDING AND DESCENDING REFLEX MOTOR ACTIVITY

Neli Nedialkova*, Galya Stavreva**, Negrin Negrev***,
Radomir Radomirov*,**

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Abstract

Electrically-elicited ascending and descending motor responses of longitudinal and circular muscles in experimental model of isolated segments of rat colon were recorded to characterize the involvement of orally and anally directed reflex pathways in underlying the motility of colonic region of the large intestine. Rectangular electrical stimuli (0.8 ms, 40 V, 5 Hz, 20 s) applied to the middle part of the segment-preparations placed in three-compartment organ bath evoked tetrodotoxin (0.1 μ M)-sensitive ascending contractions in the longitudinal and circular muscles of the proximal part of the colonic segments that were more pronounced as compared to the descending contractions of the longitudinal muscle and relaxation followed by contraction in the circular muscle belonging to the distal region of the preparations. The results indicate that locally-applied electrically-induced excitation activates synchronously by one and the same stimulus ascending and descending motor reflex pathways and characterizes the excitatory ascending motor pathways as nerve structures dealing essential role in the contractile potency of both longitudinal and circular muscles as compared to the inhibitory ability of the descending motor pathways supply the circular muscle.

Key words: ascending motor reflexes, descending motor reflexes, circular muscle, longitudinal muscle

Introduction. The distal part of the gut is a target for diseases with medical and healthy impact that could alter the peristaltic reflexes underlying the colo-rectal motility. The peristalsis of colon is closed with the movements of rectum, and colo-rectal stimulatory [1], recto-colonic inhibitory [2-4] motor activity has been demonstrated. The spontaneous motor activity is rhythmically occurring

neurally mediated motor pattern [5] exhibiting irregular high amplitude propagated contractions [6]. Despite the knowledge on the motor activity of the large intestine, the physiological significance of colon has advanced considerably, and rectum as storage or conduit organs is not defined [7] and the role of the nerve excitation-induced motor responsibility of the longitudinal and circular muscles in the coordinated motor activity of colo-recto anal region is not fully understood. Longitudinal and circular muscles expressed synchronous contractile movements in the guinea pig distal colon [8], while reciprocal activity of both muscles was demonstrated in rat colon [9]. Recent observations have demonstrated triggering of the polarized neural reflexes in murine large intestine by longitudinal stretch [5] and essential role of longitudinal muscle in ascending reflex contractility in rat colon [10], while in the guinea pig distal colon the ascending excitatory and descending inhibitory neuronal pathways are critically dependent on sensory elements within the circular muscle layer [11]. Obviously, the role of reflex nerve networks underlying contractile and/or relaxant activity of both muscles requires further elucidation.

In the present study, we have re-examined reflex-mediated motility of colon using segment-preparations isolated from rat as experimental model. In particular, we were interested in comparison of motor events and potency of both muscles as a display of excitation of reflex nerve pathways controlling the ascending and descending motor activity. Partitioned three-compartment organ bath, electrical field stimulation and mechanographic on-line technique were used in the experimental programmes.

Materials and methods. The experiments were carried out in the Laboratory of Peripheral Synapses of the Institute of Neurobiology, Bulgarian Academy of Sciences in accordance with the Ethics Committee of the Institute of Neurobiology.

Animals and isolated preparations. 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 and mounted horizontally in triple organ bath [10].

A modification of the partitioned organ bath method for studying reflex motor responses in isolated intestinal segments was used [12]. The flat 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.

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 preparations was simultaneously registered [13].

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 (Laboratorni pristroje, Prague, Czech Republic).

Electrical stimulation. Electrical field stimulation [14] (EFS) was used to excite the nerve structures of the isolated segments. EFS (0.8 ms, 40 V, 5 Hz, 20 s) was applied by means of two platinum electrodes (0.45 mm thick) placed diametrically opposed along the sides of the middle compartment at a separation of 14 mm at interval not less than 5 min [12]. The stimulation of the middle part of segments placed in the middle compartment of the bath elicited responses of both muscles belonging to proximal and distal part of colon (situated in the oral and anal compartments respectively) at a distance of 10 mm. These responses were considered as “ascending or descending” responses due to propagation of excitation via orally or anally directed reflex pathways. The motor responses elicited by electrical stimulation applied at a frequency of 5 Hz resembled by pattern and amplitude to the spontaneous high-amplitude contractions [13] and were considered as suitable for comparison of responsiveness of both muscles.

The spontaneous muscle tone of the preparations in the longitudinal and circular axis was considered as a baseline for measuring the magnitude of motor responses as force in mN. Data are presented as mean values $\pm$ S.E.M. Statistical significance was assessed by Student's *t*-test for paired and unpaired data at $p < 0.05$ using computer programmes.

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, Zurich, Switzerland) was used to verify the nerve-mediated nature of the electrically-elicited responses. The drug was dissolved in distilled water, diluted to the final concentration in the Krebs solution before use and administered in volumes not exceeding 1% of bath volume. The stock solution of TTX was stored at –18 °C.

Results. As it was already shown [10], the isolated segments of the rat colon possessed spontaneous motor activity characterized by high-amplitude irregular contractions of both longitudinal and circular muscles that propagated from the proximal to the distal part of the isolated preparations.

The application of EFS to the middle part of the colonic segments elicited

ascending motor responses of the longitudinal and circular muscle of the proximal part and descending motor responses in both muscles belonging to the distal part of the preparations at a distance of 10 mm. The electrically-elicited motor activity appeared synchronously in both proximal and distal parts of the colonic segments.

The ascending motor responses in longitudinal and circular muscles of the proximal part of colon were contractions which persisted during the whole time of electrical stimulation. The ascending responses in both muscles were similar by pattern. The ascending contractions of the longitudinal muscle were considerably higher by amplitude than the contractions of the circular muscles (longitudinal muscle 14.1 ± 1.3 mN and circular muscle 4.2 ± 0.5 mN; $n = 12$; $p < 0.05$ respectively) (Fig. 1A).

The descending motor responses of the longitudinal and circular muscles belonging to the distal part of colon differed by pattern. The longitudinal muscle responded with contractions with a pick of 6.6 ± 0.8 mN ($n = 10$) while the response of the circular muscle consisted of initial relaxation of 1.8 ± 0.3 mN followed by contraction of 3.8 ± 0.5 mN ($n = 10$). The descending contraction of the longitudinal muscle significantly exceeded the contractile component of the descending motor response of the circular muscle ($p < 0.05$) (Fig. 1B).

Discussion. The present electrically-elicited ascending and descending motor responses of colonic longitudinal and circular muscles were neurogenic by nature since they were not observed in the presence of TTX, a blocker of neuronal conductance. The triple organ bath used in the experiments allowed simultaneous recording of motor activity from the proximal and distal parts of colonic segments due to activation of nerve pathways involved in colonic integrative circuitry.

Electrical stimuli applied to the middle part of the colonic segments induced simultaneous ascending and descending motor responses in proximal and distal parts of colon. These observations indicate that the locally induced nerve excitation propagated via enteric nerve structures and demonstrate that ascending and descending reflex pathways in colonic experimental model could be synchronously co-activated by the one and the same stimulus.

The ascending motor responses of both muscles and the descending motor responses of longitudinal muscle were contractions. An initial relaxation appeared in the descending motor responses of the colonic circular 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 colonic motility relies on the activation of ascending excitatory and descending inhibitory nervous pathways to circular muscle [8, 15].

The electrically-elicited contractile ascending or descending responses of the longitudinal muscle were considerably more pronounced than those of the circular muscle suggesting an essential role of longitudinal muscle in the colonic motility. The difference in the responsibility could be related to specific organization of neuro-neuronal and/or neuromuscular communications of both muscles. The con-

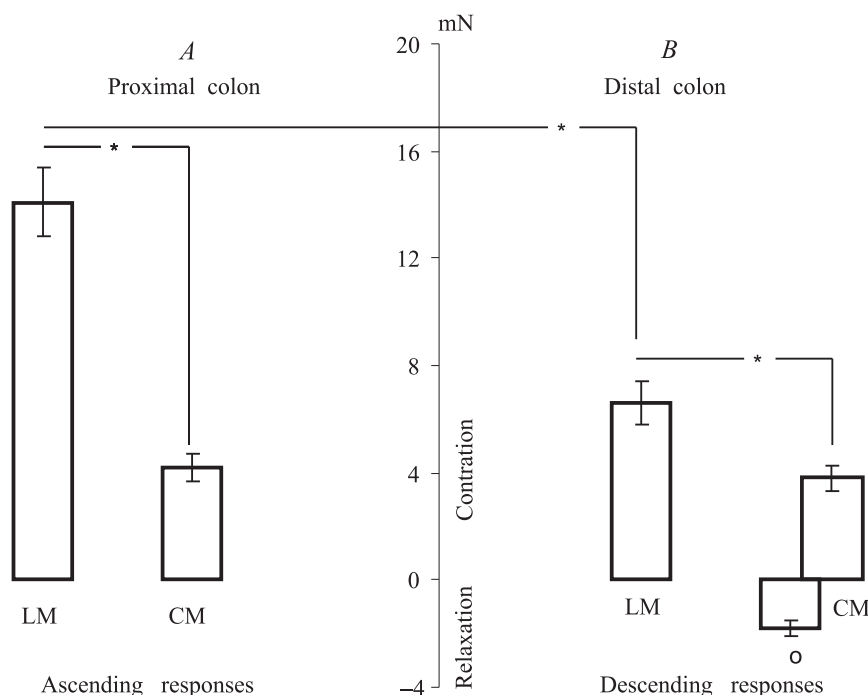


Fig. 1. Electrically-elicited (0.8 ms, 40 V, 5 Hz, 20 s) ascending motor responses of the longitudinal (LM) and circular (CM) muscles recorded from the proximal part of isolated segments from the rat colon (A) and descending motor responses of the longitudinal (LM) and circular (CM) muscles recorded from the distal part of isolated segments from the rat colon (B). The values represent means $\pm$ S.E.M. Symbols indicate: significant differences at $p < 0.05$ (*) and opposite effects (o)

tractility of colonic longitudinal muscle is mainly dependent on cholinergic and tachykininergic motor neurons releasing excitatory neurotransmitters [9] while the release of the inhibitory neurotransmitter nitric oxide to nerve stimulation in colon is greater in the circular than in longitudinal muscle [16]. We observed that the ascending contractions of longitudinal and circular muscles were more pronounced suggesting that in the isolated colonic segments the propagation of excitation was expressed more in ascending than in descending reflex pathways.

In summary, electrical stimuli applied to the middle region of isolated segments of the rat colon elicit simultaneous motor responses of the longitudinal and circular muscles belonging to the proximal and distal parts of the colon that demonstrates co-activation of excitatory and inhibitory reflex pathways underlying ascending contraction in both muscles and descending relaxation in the circular muscle. The longitudinal muscle and ascending motor responses express higher contractile potency suggesting their essential role in the coordinated colonic motor motility.

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**Institute of Neurobiology
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 23
1113 Sofia, Bulgaria*

***Department of Experimental
and Clinical Pharmacology
Medical University of Pleven
1, Kl. Ohridski Str.
5800 Pleven, Bulgaria
e-mail: drstavreva@yahoo.com*

****Department of Physiology and Pathophysiology
Medical University of Varna
Marin Drinov Str.
9000 Varna, Bulgaria*