

CHOLINERGIC, TACHYKININERGIC AND NITRERGIC
TRANSMISSION IN ASCENDING MOTOR ACTIVITY
OF COLONIC LONGITUDINAL MUSCLE IN A RAT MODEL

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Abstract

The purpose of the present study was to examine in vitro the motor activity of longitudinal muscle (LM) based on local neuronal circuitry and ascending reflex pathways. The electrically-induced local and ascending motor responses of rat colonic LM were studied using partitioned three-compartment organ bath, electrical field stimulation (0.8 ms, 40 V, 5 Hz, 20 s) and mechanographic on-line recording techniques. To establish the role of excitatory and inhibitory neurotransmissions in the reflex pathways, the motor activity was studied by cholinergic-, tachykininergic- and nitrergic-related drugs. Atropine (0.3 μ M) added in the oral compartment of the bath considerably decreased the amplitude of ascending contractions of the LM provoked by electrical stimulation (5.4 ± 0.6 mN, $n = 10$, $p < 0.05$). During atropine treatment spantide (0.1 μ M) further significantly suppressed the ascending contractile motor responses (3.2 ± 0.3 mN, $n = 8$, $p < 0.05$). L-NNA (0.5 mM), an inhibitor of nitric oxide synthase increased the contractions during atropine treatment, while L-arginine (0.5 mM) decreased the amplitudes of contractile responses. Atropine inhibited the ascending contractile responses of the LM of colonic segments indicating the essential stimulatory role of the cholinergic system in the colonic contractility. The present experiments also demonstrated the involvement of tachykininergic and nitrergic neurotransmission in the ascending motor responses of the colonic LM.

Key words: L-arginine, ascending motor response, atropine, NG-nitro-L-arginine, rat colon, spantide

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Introduction. Colonic motility is an object of experimental and clinical studies because of the high medical and social impact of nerve-mediated diseases in the large intestine [1]. The interstitial pacemaker cells of Cajal and the enteric nervous system are critical for normal motility control [2,3]. Experimental data have indicated that intrinsic pacemaker mechanisms responsible for generation and propagation of the colonic motor complexes are preserved in in vitro conditions, although the transit along the colon in vivo could be impaired [4-6]. The physiological significance of colon as storage and conduit organ is not clear yet and the neuronal mechanisms underlying propulsion of colonic content are not completely understood [3,7]. The specific role of either longitudinal or circular muscle in the reflex motor activity is debatable [8-10]. In rat colon, an essential role in ascending reflex contractility was attributed to the longitudinal muscle [4].

The aim of the present study was to examine the motor activity of longitudinal muscle based on local neuronal circuitry and ascending reflex pathways. Partitioned organ bath allowing the application of electrical stimulation to the distal part of the isolated segment-preparation and registration of the ascending motor responses of longitudinal muscle in the proximal part of the preparation was used. To clarify the role of excitatory and inhibitory neurotransmissions in the reflex pathways, the motor activity was pharmacologically analyzed by cholinergic-, tachykininergic- and nitrergic-related drugs.

Materials and methods. The study was carried out in the Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, and Pharmacophysiology Laboratory, Medical University of Pleven. The experiments were approved by the Bulgarian Food Safety Agency (Protokol No 36/18.06.2015). The experimental protocol was conducted in compliance with the national laws and policies and in conformity with the international guidelines (EEC Council Directive 86/609, IL 358, 1, December 12, 1987).

Animals and preparations. The study was carried out on 30 male Wistar rats (weight 250–300 g), purchased from the Research and Laboratory Animal Breeding, Slivnitsa (Bulgaria). The animals were kept at a temperature of $22 \pm 2^\circ\text{C}$ and humidity of $50 \pm 10\%$, given normal pelleted diet and water ad libitum. The rats were fasted overnight, stunned by a blow on the neck and decapitated. The abdominal cavity was opened and the distal part of the large intestine was removed. A 50–55 mm long section of colon with intact nerve plexuses and smooth muscle layers integrity was isolated [11].

Experimental equipment. A partitioned organ bath for studying the motor responses in isolated intestinal preparations was used [12]. Briefly, the organ bath was divided into two parts – oral and anal compartments. The segment was mounted horizontally with the proximal and distal ends of the preparation lying in the oral and the anal compartments, respectively. The proximal part of the colonic segments was tied by a thread to strain gauges to measure the motor activity in the longitudinal axis at an initial load equivalent to 10 mN. Electrical

field stimulation (EFS) was used to excite the nerve structures of the isolated segments [13]. EFS (0.8 ms, 40 V, 2 or 5 or 10 Hz, 20 s) was applied by means of two platinum electrodes (0.45 mm thick), placed diametrically opposite each other and 14 mm apart along the sides of organ baths at an interval of at least 5 min [12]. The mechanographic on-line recording techniques were completed by strain gauges and amplifiers (Microtechna, Prague, Czech Republic), stimulators (Experimetria, Budapest, Hungary) and recorders TZ 4620 (Laboratorni pristroje, Prague, Czech Republic).

Protocol design, substances and solutions. The colonic preparation was allowed to equilibrate for 30 min before starting the experiments. The application of EFS in the oral compartment of the bath elicited motor responses of the longitudinal muscle from the oral part of colonic segments. These responses were considered as “local” motor responses due to excitation of local nerve networks lying in the field of stimulation [11]. The stimulation of the distal part of the segments elicited responses of the longitudinal muscle belonging to proximal colon at a distance of 20 mm away from the application of the electrical stimuli. These responses were considered as ‘ascending’ motor responses due to propagation of excitation via orally directed reflex pathways supplying the longitudinal muscle. The electrically-induced motor responses induced by EFS, applied at a frequency of 5 Hz, were evaluated before and after drug treatment. Drugs were added in a volume not exceeding 0.5–1.0% of the compartment volume. The contact time duration of drug treatment and the concentration of drugs were as follows: tetrodotoxin (0.1 μ M, 10 min), atropine (0.3 μ M, 15 min), spantide (0.1 μ M, 15 min), NG-nitro-L-arginine (0.5 mM, 15 min) and L-arginine (0.5 mM, 15 min). When the substances were added sequentially (atropine plus spantide or atropine plus NG-nitro-L-arginine or atropine plus L-arginine) the time course of drug action was 30 min. Modified Krebs solution was used: NaCl 120 mM, KCl 5.9 mM, NaHCO₃ 15.4 mM, NaH₂PO₄ 1.2 mM, MgCl₂ 1.2 mM, CaCl₂ 2.5 mM and glucose 11.5 mM. The solution (with pH 7.2) was constantly aerated by 95% O₂ and 5% CO₂ at 36.5 °C. Substances used were: tetrodotoxin (TTX, Sankyo, Zurich, Switzerland), atropine sulfate (Merck, Darmstadt, Germany), [D-Arg¹, D-Trp^{7,9}, Leu¹¹]-Substance P (Spantide), NG-nitro-L-arginine (L-NNA) and L-arginine (Sigma Chemicals, St. Louis, MO, USA). Drugs were dissolved in distilled water and diluted to their final concentration in Krebs solution *ex tempore*. The stock solutions of TTX and spantide were stored at –20 °C.

Statistical analysis. The lowest point of the amplitudes of spontaneous contractions was considered as baseline for measuring the amplitude of the electrically-elicited motor responses as force in mN. Data are presented as mean values $\pm$ S.E.M. and were tested by one-way ANOVA, followed by the LSD as a post-hoc test. A level of $p < 0.05$ was considered significant. All analyses were performed using Statgraphics Plus 4.1 for Windows, SPSS 14 statistical softwares.

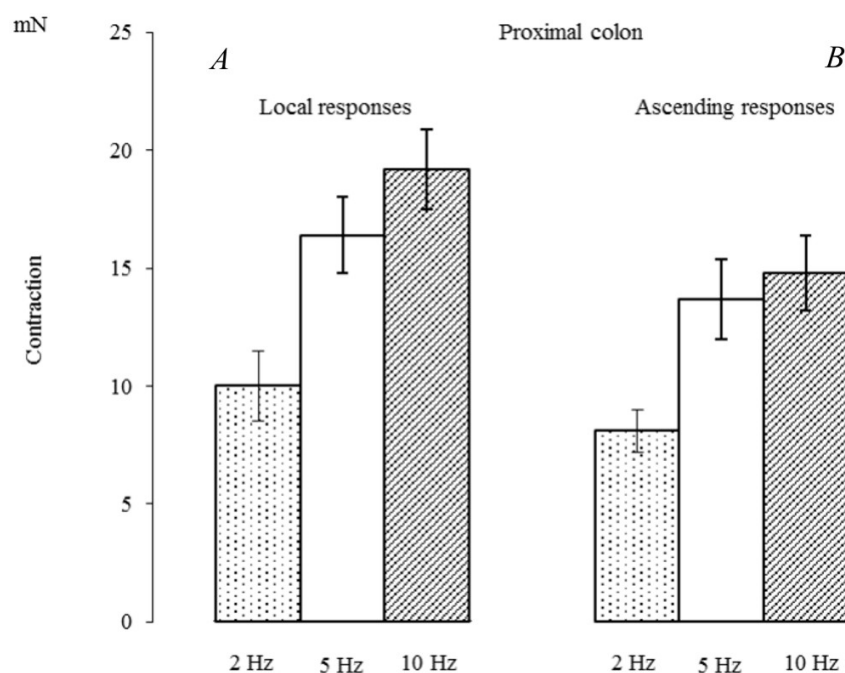


Fig. 1. Graph showing the electrically-induced (0.8 ms, 40 V, 20 s, 2, 5 or 10 Hz) local (A) and ascending (B) motor responses of longitudinal muscle belonging to the proximal part of isolated rat colonic segment

Results. The spontaneous motor activity of longitudinal muscle of isolated colonic segments represented contractions with rather stable frequency and amplitude. The muscle tone was not significantly changed for periods longer than 120 min.

Electrically-induced local motor responses. The application of EFS to the oral part of the colonic segments induced local motor responses of the longitudinal muscle. The local responses were frequency-dependent contractions which declined during the time-course of the application of the electrical stimuli. The contractions of the longitudinal muscle induced by 2-Hz electrical stimulation were significantly less pronounced as compared to the responses elicited by EFS at frequencies of 5 Hz or 10 Hz (Fig. 1A).

Electrically-induced ascending motor responses. The application of EFS to the distal part of the colonic segments caused ascending motor responses of the longitudinal muscle belonging to the proximal part of the segments at a distance of 20 mm. The ascending motor responses were frequency-dependent contractions and their pattern and amplitude were similar to those of local ones (Fig. 1B).

Drug effects on the electrically-induced ascending motor responses. Atropine (0.3 μ M) added to the nutrient solution in the oral compartment of the

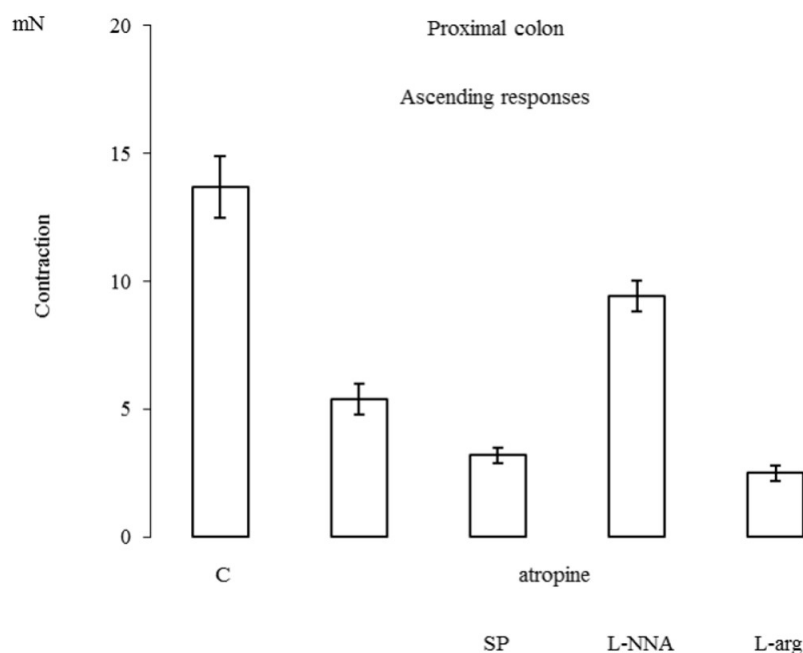


Fig. 2. Graph showing the effect of atropine, spantide (SP), NG-nitro-L-arginine (L-NNA) and L-arginine (L-arg) on electrically-induced (0.8 ms, 40 V, 5 Hz, 20 s) ascending motor responses of longitudinal muscle belonging to the proximal part of isolated rat colonic segment

bath considerably decreased the ascending contractions of the longitudinal muscle from the proximal part of colonic segments elicited by EFS applied at a frequency of 5 Hz (5.4 ± 0.6 mN, $n = 10$, $p < 0.05$ vs control ascending responses). During atropine treatment NK1 antagonist spantide ($0.1 \mu\text{M}$) further significantly suppressed the ascending contractions (3.2 ± 0.3 mN, $n = 8$, $p < 0.05$). In the presence of atropine L-NNA (0.5 mM) restored to a great extent the ascending motor responses of the longitudinal muscle while the later contractions were strongly depressed after the addition of L-Arginine (0.5 mM) (Fig. 2). The addition of TTX ($0.1 \mu\text{M}$) for 10 min ($n = 3$) to proximal and distal part of the colonic segments of the preparations abolished the local and ascending motor responses of the longitudinal muscle, respectively (data not shown).

Discussion. This study evaluated motor activity in isolated colonic segments with intact nerve plexuses and smooth muscle layers.

We observed that the application of electrical stimulation to the proximal part of the colonic segments induced local contractile motor responses of longitudinal muscle. When the distal part of the segments was subjected to EFS contractile ascending motor responses were registered in proximal part of the preparations. The motor responses induced by EFS were neurogenic by nature since they were prevented by TTX, a blocker of the sodium channel and neuronal

conduction. The increase of the frequency of EFS enhanced the amplitude of the local and ascending contractions without changing the motor pattern. It is known that more nerve terminals are involved in the process of the neurotransmitter release when electrical stimuli are applied at higher frequencies [14]. Our finding that the magnitude of electrically-elicited ascending responses was less expressed than electrically-induced local responses supports the view that the propagation of electrically-evoked excitation in the nerve structures depends on the distance from the application of stimulation [15].

Drugs were used in the present experiments to characterize neurotransmitter systems underlying the ascending motor activity of the colonic longitudinal muscle. The neuronal mechanisms that trigger peristalsis and propulsion of colonic contents are incompletely understood [3]. Atropine inhibited the electrically-induced ascending contractile responses of the longitudinal muscle of colonic segments indicating the essential stimulatory role of the cholinergic system in the colonic contractility. This observation was not unexpected. Cholinergic neurotransmission is the main factor providing the excitatory events in the intestinal motor activity. The modulation of the excitatory ascending motor pathways in the colonic region of the large intestine is a matter of debate. Activation of cannabinoid CB (1) receptors affected the cholinergic neurotransmission in mouse colon reducing the amplitude and spatial spreading of the ascending electrophysiological impulses [16]. In the rat colon, during the peristaltic reflexes, the motor activity is regulated by excitatory VIP/PACAP/NOS interneurons coupled to the cholinergic or tachykinin motor neurons innervating the longitudinal muscle [2,3]. On the background of atropine, tachykinin antagonist spantide decreased more the electrically-evoked ascending contractions of the longitudinal layer, thus preventing an excitatory action of Substance P. Most probably, tachykinin NK-1 receptors that are the main receptors mediating the effects of Substance P in the enteric nervous system [17] are involved in the ascending pathways of rat proximal colon, as it was proposed in the rabbit isolated distal colon [18].

The ascending contractile motor responses of the colonic longitudinal muscle were affected by nitric oxide-related drugs. L-NNA, an inhibitor of nitric oxide synthase increased the contractions during atropine treatment while L-arginine, a substrate of nitric oxide synthesis decreased the contractile activity. These observations support the view that the inhibitory neurotransmitter nitric oxide [19] could modulate the transmitter release and effects of other messengers in the enteric nervous system. Substance P-mediated neurotransmission is affected by nitric oxide in the small intestine [20] and a similar action of nitric oxide on the Substance P-dependent ascending contractions of rat colonic longitudinal muscle could be assumed.

The present experiments demonstrated the involvement of cholinergic, tachykininergic and nitrergic neurotransmission in the ascending motor responses of the colonic longitudinal muscle.

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