

LOCAL NERVE NETWORKS-MEDIATED ACTIVITY
OF COLO-RECTAL LONGITUDINAL AND CIRCULAR
MUSCLES IN RAT MODEL

Lidiia Simeonova, Galya Stavreva*, Neli Nedialkova,
Negrin Negrev**, Boris Kadinov, Radomir Radomirov

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Abstract

Mechanographic on-line technique and electrical field stimulation (0.8 ms, 40 V, 2 Hz, 20 s) were used to evaluate the motor responses of longitudinal and circular muscles isolated from rat colon and rectum as model preparations to display excitation of local enteric nerve networks in the large intestine. The local responses of longitudinal muscle strips isolated from proximal and distal parts of the colon and rectum were contractions. There were no differences between the peak amplitude in the motor responses of the longitudinal muscles which belonged to the colon or to the rectum. The modular contractile responses of the circular muscle rings from both parts of the colon and the middle part of the rectum were significantly less pronounced than the local responses of the longitudinal muscle strips. The electrically-induced responses of the colonic and rectal muscles were tetrodotoxin (0.1 μ M)-sensitive. The results show that low frequency-sensitive excitatory neurotransmission more expressed to the longitudinal muscle dominates in the local nerve networks of the rat colon and rectum suggesting an essential role of colo-rectal contractile mechanisms in propelling the gut content in anal direction.

Key words: local nerve networks, colon, rectum

Introduction. Because of disorders in the propulsive function of the large intestine the motor activity of the colo-rectal region is a matter of increasing medical and social interest. Complex evacuation mechanisms involve voluntary

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control of excretion and autonomic innervation of the colo-recto-anal muscles. The autonomic nerve networks are located in the gut wall since the motor events of the mammalian large intestine are presented in isolated preparations [1-4].

Enteric nerve structures form continuous overlapping networks. As small rings of the circular muscle can contract independently, the rings and the associated enteric neurons can be regarded as functional modules. The spatiotemporal coordination of the modules is a factor for the generation of rich repertoire of motor patterns [5, 6]. Despite that knowledge on the nerve-mediated motility of the large intestine has advanced, the motor activity of longitudinal and circular muscles determined by local networks in the colo-rectal region is not clear. The specific role of both muscles in spontaneous contractility and the evoked responses have not been defined.

In the present experiments, we reexamined the colo-rectal motor activity using model preparations isolated from rat. The properties of motority in the rat large intestine have scarcely been investigated [7]. In particular, we were interested in evaluating the motor responses of longitudinal and circular muscles of the colon and rectum as a display of excitation of local nerve networks controlling the motor activity. Electrical field stimulation and mechanographic on-line technique were used to evaluate the evoked motor events.

Material and methods. The experiments were carried out in the Laboratory of Peripheral Synapses of the Institute of Neurobiology at the Bulgarian Academy of Sciences and were approved by the Ethics Committee of the Institute of Neurobiology.

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 a segment consisting of colon and rectum was removed and placed in modified Krebs solution at room temperature. The extrinsic blood vessels and nerves along the mesenteric border were trimmed away. Strips, 2 mm in width and 18–20 mm in length, were isolated from the proximal and distal colon and rectum in the longitudinal axis. Rings, 2 mm in width, were dissected in the circular axis from the proximal and distal parts of colon and the middle part of the rectum. All preparations were placed in organ baths and were stretched under initial tension of 10 mN [8]. Computerized mechanographic on-line technique was used for registration of the motor activity of the isolated longitudinal muscle strips and circular muscle rings.

To excite the local nerve networks in the isolated preparations, electrical field stimulation [9] was applied by means of two platinum electrodes (0.45 mm thick) placed diametrically opposed along the sides of the organ baths at a separation of 14 mm. Rectangular pulses with a duration of 0.8 ms and voltage of 40 V were delivered at a frequency of 2 Hz for 20 s at an interval not less than 5 min [8].

The composition of the modified Krebs 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. The drug used was tetrodotoxin (TTX, Sankyo, Zurich, Switzerland). The stock solution of TTX was stored at –18 °C. The drug was diluted in Krebs solution before use. The drug was administered for 10 min in volume not exceeding 0.5–1% of the bath volume.

The spontaneous muscle tone of the preparations 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 $p < 0.05$. Calculations were made using computer programmes.

Results. The longitudinal muscle strips and the circular muscle rings isolated from rat colon and rectum responded to the electrical stimulation with fast developing twitch-like contractions. The amplitude of the electrically-induced contractile responses of the longitudinal muscle strips belonged to the proximal or the distal part of the colon and did not differ considerably. The later responses were significantly more pronounced as compared to the responses of the circular muscle rings isolated from different parts of the colon being at the proximal part of the colon 14.5 ± 1.3 mN for the longitudinal muscle vs. 7.9 ± 1.3 mN for the circular muscle ($n = 11$, $p < 0.05$) (Fig. 1) and at the distal part of the colon 16.7 ± 1.4 mN for the longitudinal muscle vs. 7.9 ± 1.1 mN for the circular muscle ($n = 11$, $p < 0.05$) (Fig. 2).

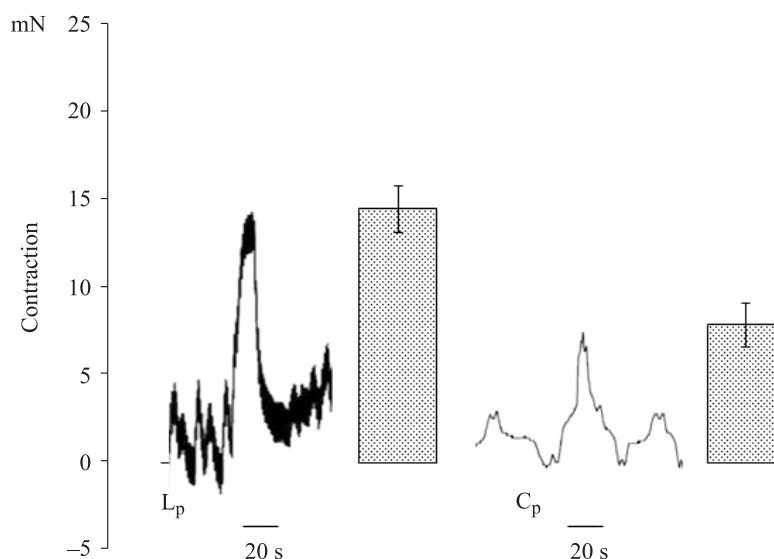


Fig. 1. Rat colon. Typical mechanographic records and histograms showing electrically elicited (0.8 ms, 40 V, 2 Hz, 20 s) motor responses of longitudinal (L_p) and circular (C_p) muscles of the proximal colon

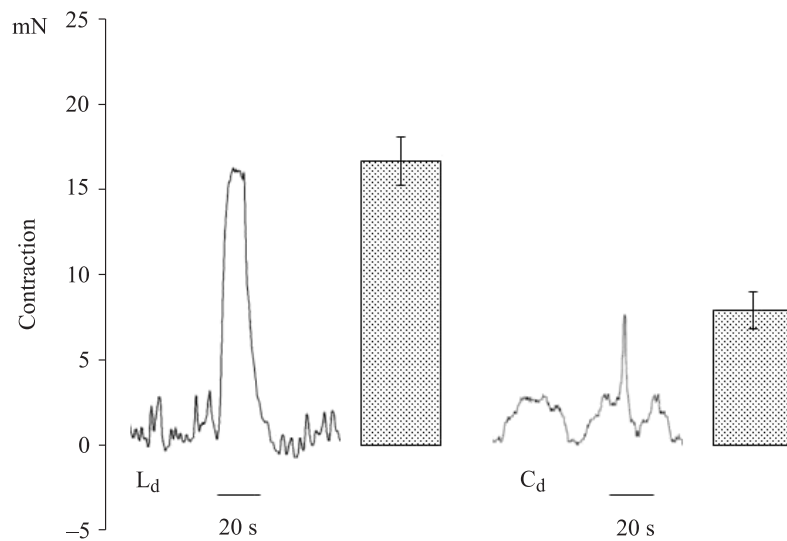


Fig. 2. Rat rectum. Typical mechanographic records and histograms showing electrically elicited (0.8 ms, 40 V, 2 Hz, 20 s) motor responses of longitudinal (L_d) and circular (C_d) muscles of the distal colon

The difference between the magnitude of electrically-elicited contractile responses of the longitudinal and circular muscles increased when these muscles were dissected from the rectum as the amplitudes were 16.2 ± 1.5 mN and 4.4 ± 0.75 mN

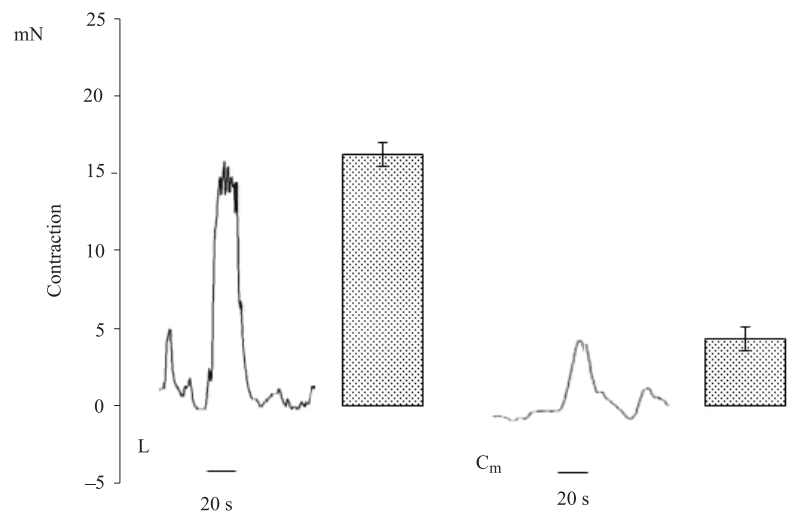


Fig. 3. Rat colon. Typical mechanographic records and histograms showing electrically elicited (0.8 ms, 40 V, 2 Hz, 20 s) motor responses of longitudinal (L) and circular (C_m) muscles of the rectum

($n = 11$, $p < 0.05$) for the longitudinal and circular muscles, respectively (Fig. 3).

The motor responses of the isolated longitudinal muscle strips and circular muscle rings were not observed when TTX at a concentration of 0.1 μM was added for 10 min in the nutrient solution of the organ bath.

Discussion. The present electrically-elicited motor responses of the isolated longitudinal muscle strips and circular muscle rings were neurogenic in nature since they were prevented by TTX, a blocker of neuronal conductance.

The electrical stimulation induces the propagation of action potential which could cause release of stimulatory and/or inhibitory neurotransmitters [9, 10]. Thus, the effect of the electrical stimulation on the intestinal muscles results in contraction or relaxation. We observed that longitudinal muscle strips and small rings of circular muscle with the associated enteric neurons that form functional modules [6] in the colon and rectum contracted in response to electrical stimulation. The responses of the longitudinal strips or circular rings isolated from the different parts of the colon and rectum were similar by pattern suggesting the release of the same excitatory neurotransmitters from the local nerve networks supplying either the proximal or the distal part of the large intestine.

The electrically-elicited contractions of the longitudinal muscle strips isolated from the proximal and distal part of the colon and along the rectum were more pronounced than the contractile responses of the circular rings. This finding indicates a dense excitatory local innervation and essential role of the longitudinal muscle in the contractile activity of the colon and rectum. The role of the longitudinal and circular muscles in the motility of the large intestine and the function of the colon and rectum as conduits or as reservoirs of the gut content is not fully clear [11]. The contractile responses of the longitudinal muscle increased from the colon to the rectum while the relaxation responses of the circular muscle remained uniformed indicating contractile potency rather than relaxation ability in the rat colorectal-anal tube [12]. Our previous investigations are in accordance with later observations showing prevalence of the excitatory neurotransmitters in the rat recto-anal region [13, 14]. The higher contractility manifested by both longitudinal and circular muscles that we observed in the present experiments suggests high physiological impact of colo-rectal contractile mechanisms in propelling the gut content in anal direction.

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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
1, Kl. Ohridski Str.
5800 Pleven, Bulgaria*

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