

LOCALITY-DEPENDENT MODULAR MOTOR RESPONSES OF ANAL REGION IN RAT MODEL

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Abstract

The responses of preparations isolated in circular axis of rat anal region to electrical stimuli were followed as a display of the modular nerve network-mediated motor activity in the distal part of gut. Electrical field stimulation (0.8 ms, 40 V, 2–5–10 Hz, 20 s) elicited twitch-like frequency-dependent contractions of the circular muscle rings belonging to the distal part of rectum and the anal canal, being more pronounced in the rectal preparations. The responses of the internal anal sphincter varied depending on the frequency of the electrical stimuli, from deep relaxation induced by stimulation applied at frequency of 2 Hz to an initial short relaxation followed by a contraction evoked by a 10-Hz stimulation. Tetrodotoxin (0.1 μ M) abolished the modular motor responses. The results indicate locality- and frequency-dependent motor responses and suggest prevalence of excitatory neurotransmission(s) in the functional modules of the distal rectum and inhibitory neurotransmission(s) in the modular nerve networks of anal sphincters. The most expressed by amplitude contractile responses of the circular rings preparations from the distal rectum most probably demonstrate higher potency of rectum to overcome the resistance of the contracted anal canal.

Key words: anal region, circular axis, modular responses

Introduction. The coordinated motor activity of intestinal longitudinal and circular muscles in mammals aims at moving the gut content in the aboral

direction in vivo. It is no doubt that in vitro condition motor activity occurs in segment-preparations isolated from different regions of the intestine, thus indicating that the nervous structures mediating the motility are present within the gut wall [1–4]. The motor activity of the recto-anal region is an object of experimental and clinical interest since this region is a target of neurodegenerative processes, traumas and tumours. The recto-anal evacuation motority is a complex process depending on the voluntary control of excretion and on the autonomic enteric neuro-neuronal and neuro-muscular communications [4].

The recto-anal evacuation activity is mainly dependent on the autonomic recto-anal biphasic reflex consisting of contraction of rectal circular muscle and synchronous relaxation of internal anal sphincter, thus presenting an essential role of the motor activity in the circular axis of the recto-anal region [5,6].

The knowledge on large intestine motility advanced considerably during the last years. However, the role of local neuronal networks-mediated motor activities of rectum and anal sphincters is not clear. Small rings of intestinal circular muscle can contract independently and the rings with the associated enteric neurons could present functional modules underlying different motor events along the gut. The spatiotemporal coordination of the modules is the determining factor for the generation of the rich repertoire of motor patterns [7,8].

The aim of the present study was to reexamine the motor activity of the anal region using recto-anal preparations isolated from rat as experimental models. We evaluated the motor responses of circular muscle rings of distal rectum, internal anal sphincter and anal canal as a display of excitation of local modules of nerve networks underlying motority in the circular axis of anal region. Electrical field stimulation was applied to activate the modular nerve structures and mechanographic computerized on-line technique was used to register the electrically-elicited motor responses.

Materials and methods. The experiments were carried out in the Laboratory of Peripheral Synapses of the Institute of Neurobiology of the Bulgarian Academy of Sciences and were approved by the 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 pubic symphysis was cut away, thus exposing the large intestine. The perianal skin was excised and the rectum with the anal canal was removed and placed in modified Krebs solution at room temperature. The extrinsic blood vessels and nerves along the mesenteric border were carefully trimmed away. A segment consisting of the distal part of the rectum and the anal canal with intact nerve plexuses-smooth muscle layers was isolated [9].

Isolated preparations. Rings, 2 mm in width, were dissected in the circular axis from the distal part of the rectum. The anal canal, 3–4 mm in width, was isolated from the rectum. The motor activity of the circular muscle rings,

the internal anal sphincter (1.5–2 mm in width), after the external sphincter had been dissected off, and the anal canal were measured between two opposite sites of the ring circumference. All preparations were placed in organ baths and stretched under an initial tension equivalent to 10 mN [10,11]. A computerized mechanographic on-line technique was used for registration of the motor activity of the isolated circular muscle rings.

Electrical stimulation. To excite the nerve structures involved in the circular preparations electrical field stimulation (EFS) was used [12]. EFS was applied by means of two platinum electrodes (0.45 mm thick) placed diametrically opposed along the sides of 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, 5 or 10 Hz for 20 s at an interval not less than 5 min [10].

Analysis. The responses of the isolated rings of circular muscle of distal rectum, internal anal sphincter and anal canal elicited by EFS were considered as ‘modular motor responses’ due to excitation of the local modules of nerve networks belonging to the isolated circular preparations.

The preparations were allowed to equilibrate for 30 min before starting the experiment.

Solutions and drugs. 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 –20 °C. Drug was administered in volumes not exceeding 0.5–1% of the bath volume as the time course of treatment and the concentration of drugs were 10 min and 0.1 µM respectively [13].

Statistics. The spontaneous muscle tone of the preparations was considered as a baseline for measuring the magnitude of electrically-elicited motor responses as force in mN. Data are presented as mean values ± S.E.M. Statistical significance was evaluated by one-way ANOVA, followed by the LSD as a post-hoc test at $p < 0.05$. Calculations were made using computer programmes for pharmacological experiments.

Results. The spontaneous motor activity of circular muscle rings isolated from distal rectum of rat was characterized by irregular contractions while the internal anal sphincter and the anal canal expressed rhythmic low-amplitude phasic contractions. The tone of preparations did not show considerable changes for a period longer than 120 min.

The electrically-elicited modular responses of the preparations isolated in circular axis from the rat anal region differed by pattern. When EFS was applied at a frequency of 2 Hz the circular muscle rings belonging to the distal rectum responded with fast twitch-like contractions (Fig. 1A), the internal anal sphincter expressed a deep relaxation of 3.57 ± 0.43 mN ($n = 9$) which persisted during the

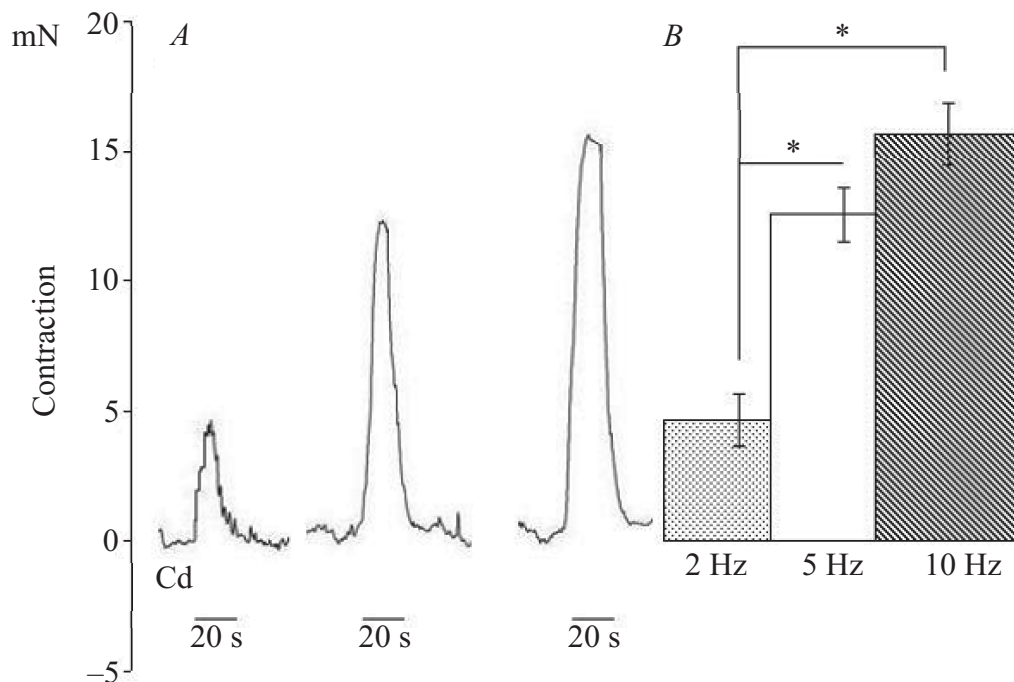


Fig. 1. Modular motor responses of circular muscle rings isolated from rat distal rectum (Cd). (A) typical mechanographic records and (B) graphs showing the responses induced by electrical stimulation (0.8 ms, 40 V, 20 s) applied at a frequency of 2, 5 or 10 Hz. Bars represent the mean $\pm$ SEM of at least 8 experiments. (*) indicates significant differences at $p < 0.05$

whole time of the electrical stimulation (Fig. 2A) and anal canal contracted by manner similar to the contractions of the rectal preparations (Fig. 3A). There was no significant difference between the amplitudes of contractile responses of the circular muscle rings from the distal rectum and the anal canal, although the contractions of rectal circular muscle rings were more pronounced (Figs 1B and 3B). The modular responses of rectal circular muscle rings were frequency-dependent. The values of contractions evoked by EFS applied at frequencies of 5 Hz (12.5 ± 1.0 mN) or 10 Hz (15.3 ± 1.2 mN) were significantly higher than those induced by 2 Hz-EFS (4.85 ± 0.48 mN, $n = 13$, $p < 0.05$) (Fig. 1B).

The modular responses of the internal anal sphincter induced by electrical stimulation applied at frequencies of 5 Hz and 10 Hz considerably differed from each other by pattern (Fig. 2A). Relaxation which was shorter by duration (Fig. 2A) and less pronounced by amplitude (3.13 ± 0.41 mN, $n = 10$, $p < 0.05$) (Fig. 2B) as compared to the relaxation obtained to 2-Hz EFS was observed when electrical stimulation of 5 Hz was used. Electrical stimuli with the frequency of 10 Hz elicited a modular response of internal anal sphincter consisting of an initial

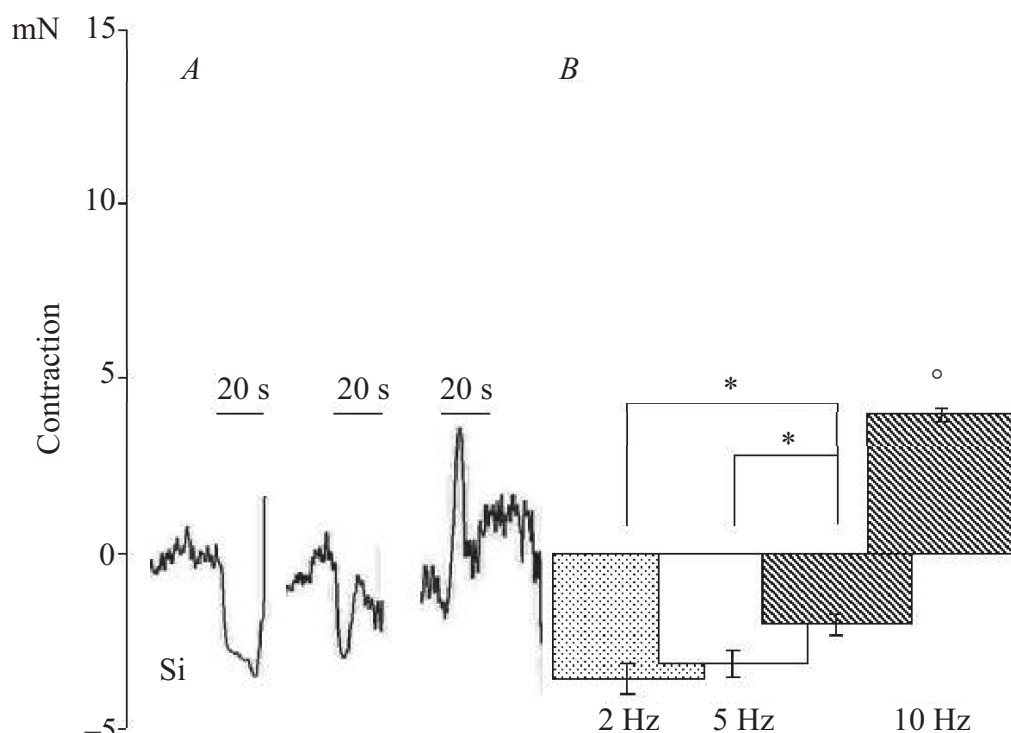


Fig. 2. Modular motor responses of internal anal sphincter (Si) isolated from rat. (A) typical mechanographic records and (B) graphs showing the responses induced by electrical stimulation (0.8 ms, 40 V, 20 s) applied at a frequency of 2, 5 or 10 Hz. Bars represent the mean $\pm$ SEM of at least 8 experiments. Symbols indicate: significant differences at $p < 0.05$ – (*) and opposite effects (°)

short-lasting relaxation followed by a contraction (Fig. 2A) reaching an amplitude of 3.95 ± 0.19 mN, $n = 10$ (Fig. 2B). The relaxation of the internal anal sphincter (2.0 ± 0.31 mN), which was observed as a component of the response to 10-Hz stimulation, was significantly less expressed than the relaxation induced by EFS applied at a frequency of 2 Hz or 5 Hz ($n = 10$, $p < 0.05$) (Fig. 2B).

The responses of the anal canal to the increase of stimulus frequency of 5 Hz or 10 Hz were frequency-dependent contractions (Fig. 3A) which were significantly less pronounced as compared to the contractions of the circular muscle rings isolated from the distal part of rectum. The most considerable difference was observed between the responses obtained when EFS was applied at frequency of 10 Hz (mean amplitude of contractions of the rectal circular muscle rings, 15.27 ± 1.22 mN vs. mean amplitude of the contractions of the anal canal, 9.24 ± 0.93 mN, $n = 13$, $p < 0.05$) (Figs 2B and 3B).

Discussion. The present study showed a modular motor activity in the circular axis of the anal region using preparations isolated from rats as experimental models. Small rings of circular preparations with the associated enteric

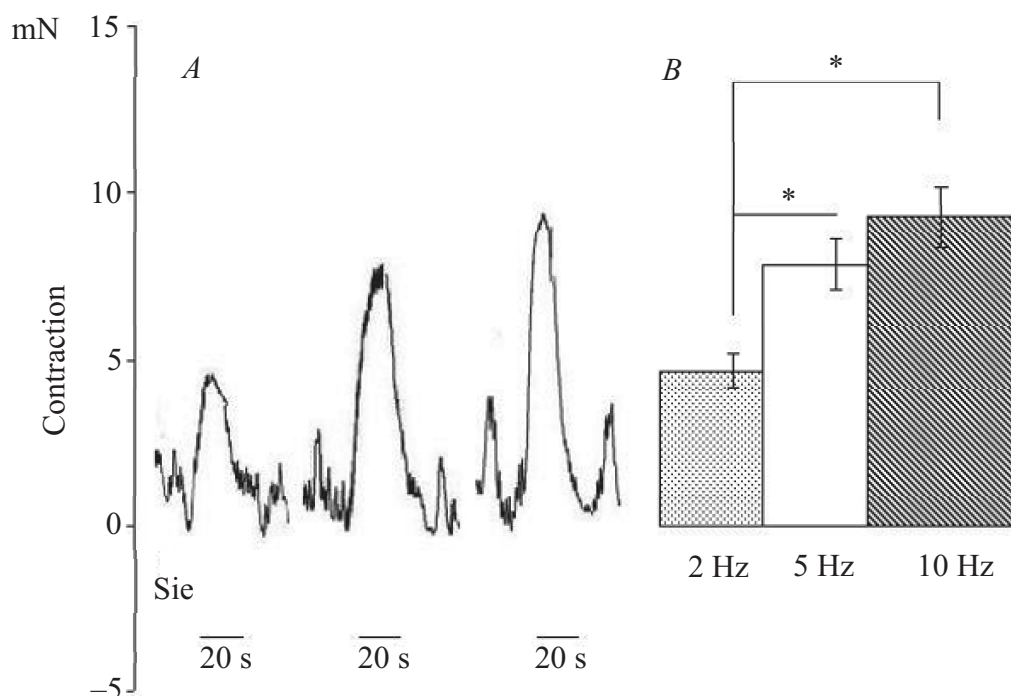


Fig. 3. Modular motor responses of anal canal (Sie) isolated from rat. (A) typical mechano-graphic records and (B) graphs showing the responses induced by electrical stimulation (0.8 ms, 40 V, 20 s) applied at a frequency of 2, 5 or 10 Hz. Bars represent the mean $\pm$ SEM of at least 8 experiments. (*) indicates significant differences at $p < 0.05$

neurons that form functional modules [8] responded to electrical stimulation. The electrically-induced motor responses of circular muscle rings of the distal part of the rectum, internal anal sphincter and anal canal were neurogenic in nature since they were prevented by TTX, a blocker of neuronal conductance.

The electrical stimuli induced propagation of action potentials in nerve terminals that resulted in local release of stimulatory and/or inhibitory neurotransmitter(s) [12,14]. We observed that the circular muscle rings belonging to the distal rectum and anal canal responded to the electrical stimulation by twitch-like contractions. The responses were frequency-dependent without changing the pattern of responses. This finding indicated that the increase of contractile responses was due to the frequency-dependent release of the same neurotransmitters since during high frequency stimulation, the total number of excited nerve varicosities involved in the process of release of neurotransmitters is greater than the number of varicosities releasing transmitter during stimulation with single pulse [15,16]. We observed that the modular contractile responses of the circular muscle rings belonging to the distal rectum exceeded by amplitude the contractile responses of the anal canal, which could suggest a physiological necessity of higher contrac-

tile potency of the distal rectum to overcome the resistance of contracted anal canal. The electrically-induced modular responses of the internal anal sphincter differed depending on the frequency of the electrical stimuli. Deep relaxation was observed as a response to lowest 2-Hz EFS while short-lasting relaxation followed by contraction appeared when highest 10-Hz stimulation was applied. Electrical stimulation could induce relaxation [17,18] or contraction [19] in internal anal sphincter. The present experiments showed that the modular neurotransmission-network supplying the internal anal sphincter involves nerve pathways sensitive to different type of excitation. Most probably, in physiological conditions, the integrity of internal and external anal sphincters and associated nerve networks act as a functional module that is involved in the coordination of the motor activity of anal canal.

In summary, it could be suggested that the different locality-dependent motor activities in circular axis of the anal region display the prevalence of stimulatory or inhibitory neurotransmitter system that determines the continuity of the phases of the evacuation process.

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