

**ELECTRICALLY- AND DISTENSION-DEPENDENT REFLEX
PATHWAYS CONTROLLING THE MOTOR ACTIVITY
OF ANAL CANAL**

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Abstract

The motor responses of anal canal in rat experimental model were followed as a display of the controlling role of descending recto-anal reflex pathways on contraction/relaxation events underlying the evacuation activity. The nerve structures in the entire recto-anal segment-preparation mounted in two-compartment organ bath were excited by electrical stimulation applied to the rectum or the anal canal and by balloon-induced distension of the proximal or the distal rectal wall. Electrical field stimulation (0.8 ms, 40 V, 5 Hz, 20 s) applied to the rectum elicited twitch-like descending contractile response of the anal canal. More pronounced contraction was observed when the electrical stimuli were applied directly to the anal canal. The motor activity of the anal canal evoked by balloon inflation in the rectum differed depending on the locality of the application of distension along the rectum. The anal canal responded by descending contraction or by short-initial contraction followed by relaxation when the distension was applied to proximal or to distal part of the rectum, respectively, indicating an essential role of mechanoreceptors in activation of long excitatory and short inhibitory descending recto-anal motor reflex pathways.

Key words: recto-anal region, descending motor reflexes

Introduction. Because of the high healthy and social impact of disorders targeting the human large intestine, the mechanisms controlling the evacuation process are a matter of experimental and clinical studies. There is no doubt now that the recto-anal nerve-mediated motility and evacuation activity are complex processes involving the voluntary control of the excretion as well as the autonomic innervation of the rectum and internal and external anal sphincters [1]. The autonomic nerve structures underlying the motor activity of intestinal smooth muscles are located in the gut wall and experimental preparations isolated from

the mammalian large intestine can display reflex contractile/relaxation events [2–4]. Functionally-different excitatory [5, 6] and inhibitory [4, 7, 8] reflex pathways have been demonstrated in the recto-anal region. The control of motor activity during evacuation is mainly attributed to the recto-anal inhibitory reflex which consists of contraction of rectum and relaxation of internal anal sphincter [1, 9, 10], highlighting the role of descending inhibitory pathways supplying anal sphincters.

Although knowledge on the reflex motility of the large intestine has advanced considerably over the last years, the functional activity of recto-anal region has remained obscure [11] and the role of recto-anal neuronal circuitry in the evacuation reflex of anal canal is not well studied [12]. In these experiments, we re-examined the motility of anal canal using model preparations isolated from rat. We were particularly interested in evaluating the motor responses as a display of the dependence of descending reflex pathways on different types of exciting stimuli applied to the rectum. Mechanographic on-line technique, a partitioned organ bath, electrical stimulation and balloon-induced distension were used in the experiments.

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 Animal Care and Use Ethics Committee of the Institute of Neurobiology.

Animals. Male Wistar 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. The perianal skin was excised and the anal canal with the 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. A segment consisting of the distal part of the rectum and the anal canal with intact nerve plexuses-smooth muscle layers was isolated [13]. The recto-anal preparation, 18–21 mm in length, was mounted in a partitioned organ bath.

Partitioned organ bath method. A modification of the partitioned organ bath method for studying reflex motor responses in isolated intestinal segments was used [14]. The flat organ bath was divided in two compartments by plastic partition with a slit filled by a paraffin “diaphragm”, thus consisting of oral and anal compartments. The recto-anal preparation was gently threaded through a 2-mm diameter hole in the paraffin diaphragm. The rectum (16–18 mm in length) was placed in the oral compartment and the anal canal (3–5 mm in length) was mounted in the anal compartment of the bath. Inert silicone grease was then applied around the gut circumference in the paraffin diaphragm to seal any possible contact between solutions in the two compartments. Motor activity of the anal canal in entire recto-anal segment preparation was measured between two opposite sites of the ring circumference, one secured to a plastic rod and the other connected to a strain gauge. Anal canal was stretched under an initial tension

equivalent to 10 mN [15]. Strain gauges (Microtechna, Prague, Czech Republic), stimulators and amplifiers (Experimetria, Budapest, Hungary) and recorders TZ 4620 (Laboratorni pristroje, Prague, Czech Republic) were used for registration of motor responses.

Electrical stimulation. Electrical field stimulation (EFS) [16] 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. Rectangular pulses with duration of 0.8 ms and voltage of 40 V were delivered at a frequency of 5 Hz for 20 s at an interval not less than 5 min [14].

The application of EFS either in the anal or in the oral compartment of the bath elicited motor responses of the anal canal. The responses to EFS applied to the anal canal in the anal compartment were considered as local responses due to excitation of nerve structures lying in the field of electrical stimulation. EFS applied in the oral compartment of the bath, where the rectal part of the entire recto-anal preparation was situated, elicited motor responses of the non-stimulated anal canal lying in the anal compartment of the bath. These responses were considered as descending responses due to propagation of excitation via rectal anally-directed reflex pathways.

Balloon-induced distension. Balloon-induced distension of the distal part of the rectum was performed by a polyethylene balloon-tipped fine plastic tubule connected to a 1 ml micro-syringe. The size of the balloon inflated by Krebs solution (stepwise, volume controlled inflation by 0.04–0.40 ml at 36.5 °C) imitated the size of rat excrements (2.5–3.0 mm in diameter). The empty balloon was pushed via the lumen of the rectum of the recto-anal preparation and was gently moved in the anal direction as inflated and deflated slowly (30 s) at a distance of 15–12 or 5–2 mm apart from the anal canal [17].

Analysis and statistics. The spontaneous muscle tone of the anal canal was considered as a baseline for measuring the magnitude of motor responses as force in mN. To evaluate the declination of excitation via reflex pathways, the descending electrically-elicited responses were compared to the local electrically-elicited responses of the anal canal. 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 for pharmacological calculations.

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.

Tetrodotoxin (TTX, Sankyo, Zurich, Switzerland) was used. The drug was dissolved in distilled water, diluted to the final concentration in 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. Electrical stimulation applied to the anal canal of the entire recto-anal preparation induced local contractile responses with peak amplitude of 14.78 ± 1.74 mN ($n = 12$) (Fig. 1A). EFS applied to the distal part of the rectum in an entire recto-anal preparation elicited descending contractions of the anal canal with maximum amplitude of 5.35 ± 0.52 mN ($n = 12$) (Fig. 1B). The descending contractions of the anal canal persisted during the period of electrical stimulation and slowly declined.

The distension of the rectum by balloon induced different by manner motor responses of the anal canal, depending on the locality of the balloon inflation along the rectum. The distension of the proximal rectum, 15–12 mm apart from the anal canal, induced contraction reaching mean amplitude of 4.04 ± 0.36 mN ($n = 7$) (Fig. 1C). The distension of the distal rectum when the balloon inflation was applied 5–2 mm apart from the anal canal evoked biphasic motor responses of anal canal consisting of a short initial contraction followed by a long-lasting deep relaxation. The amplitudes of the contraction and the relaxation were 1.62 ± 0.22 mN and 2.88 ± 0.36 mN ($n = 7$) (Fig. 1D) respectively.

The electrically-elicited or distension-induced motor responses of the anal canal were not observed when TTX in a concentration of 0.1 μ M was added to the nutrient solution (data not shown).

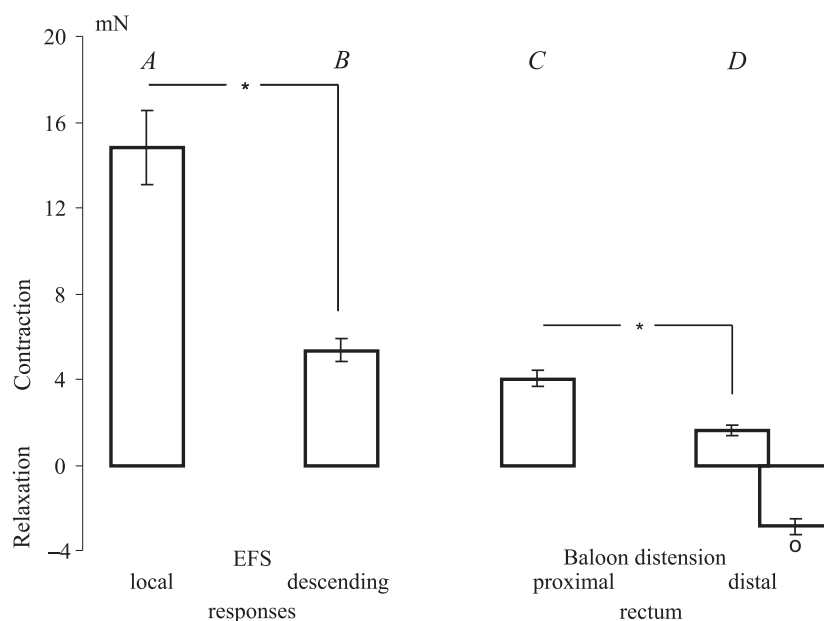


Fig. 1. Motor responses of anal canal in recto-anal preparations isolated from rat. Electrically-elicited (0.8 ms, 40 V, 5 Hz, 20 s) local (A) and descending (B) responses; balloon-induced responses at distance of 15–12 mm (C) and at distance of 5–2 mm (D). The values represent means $\pm$ S.E.M. Symbols indicate: significant differences at $p < 0.05$ (*) and opposite effects (o)

Discussion. The present electrically-elicited local and descending contractions or distension-induced descending motor responses of the anal canal were neurogenic by nature since they were prevented by TTX, a blocker of neuronal conductance. The partitioned organ bath method allowed observation of local electrically-elicited motor responses of anal canal as well as motor responses of anal canal induced by electrical stimulation or balloon distension applied in the rectum, thus characterizing different electrically- or distension-dependent descending reflex pathways involved in the recto-anal neuronal circuitry.

Electrical stimulation applied to the anal canal or to the distal part of the rectum elicited local or descending contractile responses of the anal canal. The local responses are suggested to reflect the activation of local neuro-neuronal and/or neuro-muscular communications of the anal canal area. The responses of anal canal, induced by the stimulation of the distal rectum, are considered as descending responses depending on the activation of nerve pathways along the rectum. The amplitude of electrically-elicited descending responses of the anal canal was less expressed as compared to the electrically-induced local responses. This observation is in agreement with the view that the propagation of electrically-evoked excitation in the nerve structures depends on the distance from the application of stimulation [18]. The contractile local or descending motor responses of the anal canal due to electrically-activated nerve structures suggest that mechanisms of direct excitation of recto-anal nerve structures are involved in the contractile motor events of the anal canal.

The balloon inflation-induced descending responses of anal canal depended on the locality of the application of the distension, being contraction when the proximal rectum was distended or short contraction followed by a deep relaxation when the distension was applied to the distal rectum. The later responses were opposite in pattern as compared to the electrically-elicited descending response of anal canal. The distension of the rectal wall was defined as a factor leading to excitation of mechanoreceptors. A dense afferent innervation by mechanoreceptors with specialized intraganglionic laminal endings detecting mechanical deformation and monitoring the contraction level is described in the rectum of guinea pig and cat [19–21]. Rectal wall stretch induced by rectal content could provoke the defecation reflex in humans [11]. Obviously, the activation of mechanoreceptor-dependent descending pathways located in the region of distal rectum could be responsible for the relaxation of the anal canal that we observed in rat model preparation.

Conclusion. The present study showed that electrically- and distension-dependent descending reflex pathways of the recto-anal region are involved in the control of motor evacuation activity of anal canal in a rat model preparation. A direct excitation of the recto-anal local or descending nerve structures underlies contractile potency while mechanoreceptor-dependent activation of descending reflex pathways determines relaxation events.

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