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Activation-dependent descending reflex evacuation of anal canal in a rat model

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ABSTRACT

The evacuative motor responses of the anal canal and recto-anal reflexes during defecation were studied in an isolated rat recto-anal model preparation using (i) partitioned organ bath, (ii) electrical stimulation, (iii) balloon distension and (iv) morphological techniques. Electrical field stimulation applied to the anal canal or to the distal part of the rectum elicited tetrodotoxin (10^{-7} M)-sensitive frequency-dependent local or descending contractions of the anal canal and the local responses were bigger in amplitude (14.9 ± 1.35 mN) than the descending contractions (5.3 ± 0.7 mN at frequency of 5 Hz, $p < 0.05$). The balloon-induced distension of the distal rectum evoked descending responses of the anal canal consisting of a short contraction (1.50 ± 0.18 mN) followed by deep relaxation (3.12 ± 0.34 mN). In the presence of atropine (3×10^{-7} M) the electrically-elicited (5 Hz) local or descending contractions of the anal canal were suppressed and a relaxation revealed. The initial contraction component of the distension-induced response was decreased while the relaxation was not changed. During atropine treatment, spantide (10^{-7} M) lowered even more the contractile component of the anal canal response. NG-nitro-L-arginine ($5 \times 1\ 0^{-4}$ M) enhanced the contraction, prevented the atropine-dependent relaxation of the electrically-elicited response and inhibited the distension-induced relaxation. L-Arginine (5×10^{-4} M) suppressed the contraction and extended the relaxation. ChAT-, substance P- and NADPH-diaphorase-positive perikarya and nerve fibers were observed in myenteric ganglia of the anal canal. The results suggest activation-dependent descending reflex motority of the anal canal involving electrical stimulation-displayed cholinergic and tachykininergic and distension-manifested nitrergic neuro-muscular communications.

Key words

Anal canal • Descending reflex motority

Introduction

Because of the biological and social impact of fecal continence and incontinence the motor activity of the recto-anal region is a matter of experimental and clinical significance. Recto-anal motility and evacuation are complex processes involving the voluntary control of excretion as well as the properties of smooth muscles and innervation of the rectum and internal and external anal sphincters.

The recto-anal region is regulated by a dual nerve supply: somatic and autonomic. Mechanographic and electrophysiological observations demonstrate that the motor activity of the mammalian large intestine occurs also in isolated preparations, thus indicating that localized reflex pathways underlying the motility of the distal part of the gut are contained within the gut wall (Grider and Makhlouf, 1986; Smith and McCarron, 1998; Matsufuji and Yokoyama, 2003; Bian et al., 2004; Radomirov et al., 2006).

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The physiological control of recto-anal motility during evacuation is mainly attributed to the autonomic recto-anal inhibitory reflex which consists of rectal reflex contraction and a synchronous internal anal sphincter reflex relaxation. This highlights the importance of the basal resistance offered by the internal anal sphincter (Maggi et al., 1988; Stebing et al., 1996; De Lorijn et al., 2005; Kojima et al., 2006). Despite that knowledge on large intestine motility has advanced considerably in the last years the recto-anal reflex circuitry is not fully understood. Enteric pathways form overlapping networks which can be regarded as functional modules (Costa et al., 2000). The spatiotemporal coordination of the modules is the determining factor for the generation of the rich repertoire of motor patterns (Costa and Brookes, 1994).

The modular descending reflex-mediated contractile and/or relaxant activity of internal and external anal sphincters is not clear and requires further elucidation. According to Bharucha (2006) both anal sphincters are responsible for maintenance of the neurogenic continence. We failed to find experimental data showing biological characterization of the autonomic descending nerve pathways of the rectum and the corresponding motor responses of the anal canal in the presence of preserved anatomical and functional integrity of internal and external anal sphincters.

In the present study, we have reexamined reflex evacuation activity in the recto-anal region using a recto-anal preparation isolated from rat as an experimental model. In particular, we were interested in evaluating the motor responses of the anal canal as a display of activation of modular rectal descending reflex pathways controlling the anal sphincters. Using a partitioned organ bath method the motor responses of the anal canal induced by electrical stimulation or balloon inflation applied to the distal part of the rectum were registered. To evaluate the biological role of cholinergic, tachykininergic and nitrergic nerves in the descending recto-anal reflex activity, the motor responses of the anal canal were manipulated pharmacologically by suitable agents. The presence and distribution of acetylcholine, substance P and nitric oxide in neuronal structures of the myenteric plexus of the anal canal were examined by morphological techniques. Immunohistochemistry for choline acetyltransferase (ChAT) and substance P and histochemistry for nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-diaphorase) were applied.

Methods

The experiments were carried out in the Laboratory of Peripheral synapses of 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

Thirty six 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 anal canal with the distal part of the large intestine was removed and placed in modified Krebs solution at room temperature. The extrinsic blood vessels and nerves along the mesenteric border were then 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 (Brading et al., 2008). The recto-anal preparation, 13-15 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 (Ivancheva and Radomirov, 2001). The flat organ bath was divided in two compartments by plastic partition with a slit filled by a paraffin “diaphragm”, thus consisting of an oral and anal compartment. The recto-anal preparation was gently threaded through a 2-mm diameter hole in the paraffin diaphragm. The rectum (10-12 mm in length) was placed in the oral compartment and the anal canal (3-5 mm in length) was situated in the anal compartment of the bath. The end of the rectum was tied by a silk thread to the side of the oral compartment. Motor activity of the anal canal was measured between two opposite sites of the ring circumference, one secured to a plastic rod and the other connected to a strain gauge under initial tension equivalent to 10 mN. Thus interference

from movements of the rectal part of the segment-preparation was minimized at the point of securing so that rectal motor activity caused no mechanical artifacts to responses of the anal canal. 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 (Radomirov et al., 2009a) (Fig. 1).

Electrical stimulation

Electrical field stimulation (EFS) (Paton and Vizi, 1969) was applied by means of two wire platinum electrodes (0.45 mm thick and 10 mm long) placed diametrically opposed, along the sides of the compartments and separated by a distance 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 (Radomirov and Venkova, 1988; Ivancheva and Radomirov, 2001).

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 in the anal compartment where the anal part of the preparation was placed, were considered as local responses due to excitation of nerve structures lying in the field of electrical stimulation.

EFS applied in the oral compartment where the rectal part of the preparation was situated elicited motor responses of the non-stimulated anal canal lying in the anal compartment of the bath. Later responses were considered as descending responses due to propagation of excitation via anally directed rectal 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 microsyringe. The size of the inflated by Krebs solution balloon (stepwise, volume controlled inflation by 0.04-0.40 ml at 36.5°C) imitated the size of rat faecal pellets (2.5-3.0 mm in diameter). The empty balloon was pushed via the lumen of the rectum of the recto-anal preparation. After the equilibration period the empty balloon was gently moved in the

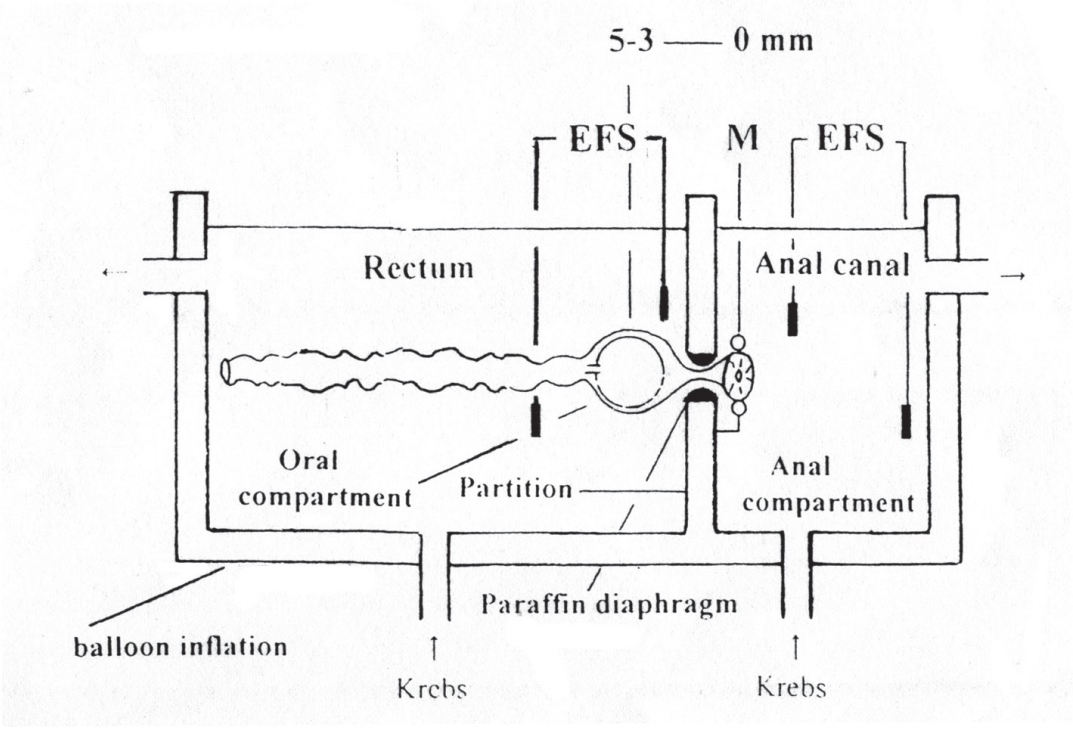

Fig. 1. - Schematic drawing of the set up for studying the electrically- or balloon inflation-induced descending reflex motor activity of anal canal in isolated rat recto-anal segment placed in a partitioned organ bath with compartments for rectum and anal canal. Designations: registration of motor activity of anal canal (M); positions of electrodes for electrical field stimulation (EFS); positions of balloon inflation at distance of 5-3 mm apart from the anal canal.

anal direction and inflated and deflated slowly (30 s) at a distance 3-5 mm away from the anal canal (Radomirov et al., 2009b).

Equipment

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.

Protocol design

The recto-anal preparation was allowed to equilibrate for 30 min before starting the experiment.
The electrically-elicited or balloon inflation-induced motor responses of the anal canal were registered before and during drug treatment. Drugs were added to the anal compartment of the partitioned bath in which the anal canal was placed and were administered in volumes not exceeding 0.5-1% of the compartment volume. The time course of drug treatment and the concentration of drugs were as follows: tetrodotoxin (10^{-7} M, 10 min), atropine (3 x 10^{-7} M, 15 min), spantide (10^{-7} M, 15 min), NG-nitro-L-arginine (5 x 10^{-4} M, 15 min) and L-arginine (5 x 10^{-4} M, 15 min). When the drugs were added consecutively (atropine plus spantide or atropine plus NG-nitro-L-arginine or atropine plus L-arginine) the time course of drug action was 30 min (Radomirov et al., 2009a).

Statistical analysis

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 decline of excitation via reflex pathways descending electrically-elicited responses were compared to local electrically-elicited responses of the anal canal. Data are presented as mean values ± 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 programs for pharmacological calculations (Tallarida and Muray, 1981).

Immunohistochemical and histochemical techniques

The presence and distribution of acetylcholine, substance P and nitric oxide in neuronal structures of the myenteric plexus of the anal canal were examined by immunohistochemistry for ChAT, substance P and NADPH-diaphorase histochemistry. Under deep anesthesia with Thiopental (50 mg/kg, i.p.) four male rats were transcardially perfused with 4% paraformaldehyde in 0.1 phosphate buffer. The recto-anal region was removed and postfixed in the same fixative solution for 24 h. Tissue sections (30 μm thick) were cut on a freezing microtome and collected in subsets of three. The first sections were processed for ChAT-, the second for substance P-reactivity by the ABC (avidin-biotin-horseradish peroxidase) method (Hsu et al., 1981) and the third subset was processed for NADPH-diaphorase (Scherer-Singler et al., 1983). The sections for ChAT- and substance P-immunohistochemistry were washed out with PBS/0.3% Triton X-100. Endogenous peroxidase was blocked with 1.2% H_2O_2 in absolute methanol for 30 min and then sections were incubated in 5% normal goat serum for 60 min. After a brief rinse in PBS, the sections were transferred for 24 h in the primary antibody solution containing rabbit polyclonal anti-ChAT, diluted 1:200. The sections for substance P were incubated in rabbit polyclonal anti-SP antibody, working dilution 1:6000, later transferred to goat anti-rabbit IgG-Biotin at a working dilution 1:250 for 90 min, and then placed in ABC complex at working dilution 1:100 for 90 min. The reaction was visualized with 3,3'-DAB/H_2O_2. The intensity of the reaction was increased with 0.05% nickel ammonium sulphate. Control sections were incubated by omission of the primary antibody and results were negative. The sections for NADPH-diaphorase were rinsed for 30 min in 1 x 10^{-4} M Tris-HCl buffer, pH 7.4 with 0.8 Triton X-100, and then incubated for 90 minutes at 37°C in 10 ml 1 x 10^{-4} M Tris-HCl buffer, pH 7.4 containing 4 mg reduced β-NADP and 10 mg nitro-blue tetrazolium. The sections were washed in PBS, mounted on chrome-gelatin-coated slides, air-dried for 24 h, dehydrated and embedded in Entellan. Control sections involved omission of substrate or the electron acceptor. The blue diaphorase reaction was eliminated in control sections.

Microscopy

The following instruments were used for microscopic studies: Reichert Jung freezing microtome (Austria), light microscope Jenaval (Germany) and light microscope with digital camera Nikon (Japan).

Solutions and drugs for motor reflex studies

The composition of the modified Krebs solution was (mM): NaCl 120, KCl 5.9, $NaHCO_3$ 15.4, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5 and glucose 11.5. The solution was continuously aerated by 95% O_2 and 5% CO_2 (pH 7.2) at 36.5°C.

Drugs used were: tetrodotoxin (TTX, Sankyo, Zurich, Switzerland), atropine sulfate (Merck, Darmstadt, Germany), [D-Arg[1], D-Trp[7,9], Leu[11]]-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 before use. The stock solutions of TTX and spantide were stored at -20°C.

Solution and drugs for morphological studies

5 x 10^{-5} M phosphate buffered saline (PBS) pH 7.3, 1 x 10^{-4} M phosphate buffer (PB) pH 7.3, 50 mM Tris-HCl buffer pH 7.4 and 7.56, Triton X-100, Hydrogen peroxyde (Fluka AG, Buch, Switzerland), Paraformaldehyde, Entellan, Methanol (Merck, Darmstadt, Germany), Normal goat serum, Reduced β-nicotinamide adenine dinucleotide phosphate (β-NADPH), Nitroblue tetrazolium chloride (NBT), 3-3'-diaminobenzidine tetrahydrochloride (3,3'-DAB) (Sigma Chemicals, St. Louis, MO, USA), rabbit polyclonal anti-choline acetyltransferase (anti-ChAT) antibody and goat anti-rabbit IgG-Biotin (Chemicon Inc., Billerica, MA, USA), rabbit polyclonal anti-substance P (Abcam, Cambridge, UK), Avidin-botin complex (Vectastain ABC kit, Vector Laboratories Inc., Burlingame, USA).

Results

Electrically-elicited local motor responses

The application of electrical stimulation at frequencies of 2, 5 or 10 Hz to the anal compartment of the bath induced local responses of the anal canal. The local responses consisted of high-amplitude contractions which declined during the stimulation (Fig. 2A). The high-amplitude contractions were frequency-dependent as responses induced by stimulation at a frequency of 5 Hz (14.9 ± 1.35 mN, n = 8) differed significantly from the responses at a frequency of 2 Hz or 10 Hz (10.1 ± 0.73 mN or 18.1 ± 1.65 mN, respectively, n = 8, $p < 0.05$) (Fig. 2B).

The local response evoked by 5 Hz-electrical stimulation was followed also in the presence of drugs. Atropine at a concentration of 3 x 10^{-7} M converted the contractile response to one consisting of initial contraction followed by relaxation. Atropine decreased the control contraction to 8.4 ± 0.6 mN (n = 8, $p < 0.05$) and induced a relaxation of 2.2 ± 0.3 mN (n = 8). In the presence of atropine, spantide (10^{-7} M) diminished the contraction further without changing the atropine-induced relaxation. L-NNA (5 x 10^{-4} M) counteracted the effect of atropine as it increased the contraction (17.1 ± 0.6 mN, n = 8, $p < 0.05$) and prevented the appearance of relaxation. L-Arginine (5 x 10^{-4} M) exerted an opposite effect by decreasing the initial contraction and considerably increasing the relaxation to 5.2 ± 0.5 mN (n = 8, $p < 0.05$) (Fig. 2C).

Electrically-elicited descending motor responses

EFS applied to the distal part of the rectum of the recto-anal preparation elicited a descending contractile response of the anal canal. The descending contraction persisted during the stimulation period and was usually characterized by phasic oscillations forming the maximum amplitude (Fig. 3A). The electrically-elicited descending responses of the anal canal were frequency-dependent as the responses to stimulation applied at a frequency of 5 Hz differed significantly from those elicited by 2-Hz or 10-Hz EFS (3.2 ± 0.4 mN or 8.1 ± 1.1 mN, n = 9, $p < 0.5$, respectively) (Fig. 3B).

The descending responses were significantly less magnitude compared to the local responses induced by the same frequency of the electrical stimulus (Figs. 2B, 3B).

Atropine (3 x 10^{-7} M) induced a slight relaxation which followed an initial contraction in the descending response induced by 5 Hz-electrical stimulation. During atropine treatment spantide (10^{-7} M) suppressed the initial contraction without altering the relaxation. L-NNA (5 x 10^{-4} M) increased the contraction while L-Arginine (5 x 10^{-4} M) significantly inhibited the contraction and increased relaxation of the atropine-sensitive descending electrically-elicited response of the anal canal (Fig. 3C).

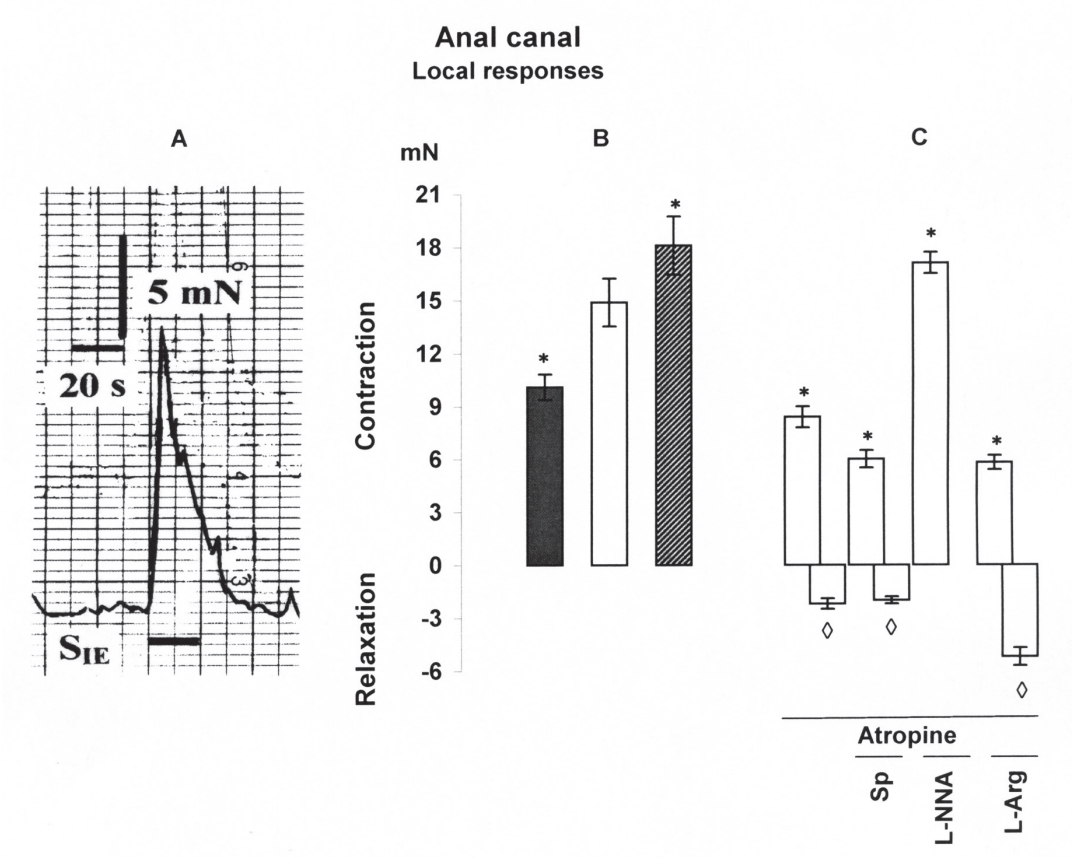

Fig. 2. - Typical mechanographic record showing electrically-elicited local motor response of anal canal (Sie) in isolated rat recto-anal segment (A); Local motor responses of anal canal elicited by EFS applied at frequencies of 2 Hz (■), 5 Hz (□) or 10 Hz (▨) (B); Local motor responses of anal canal elicited by EFS applied at frequency of 5 Hz in the presence of Atropine (3 x 10^{-7} M) or Atropine plus Spantide (Sp, 10^{-7} M) or Atropine plus NG-nitro-L-arginine (L-NNA, 5 x 10^{-4} M) or Atropine plus L-Arginine (5 x 10^{-4} M) (C). The values represent means ± s.e. mean of at least 8 experiments. Symbols indicate: significant differences at $p \leq 0.05$, 't' test ($t \geq 2.31$ at 8 experiments) - (*) drug treatment *vs.* controls and (◊) opposite effects *vs.* controls.

Balloon distension-elicited descending motor responses

The distension of the distal rectum for 30 seconds by inflation of a balloon positioned at distance of 3-5 mm apart from the anal canal induced descending motor responses of the anal canal comprised by a short contraction followed by a long-lasting deep relaxation (Fig. 4A), with amplitude of 1.5 ± 0.18 mN and 3.12 ± 0.34 mN, respectively (n = 8) (Fig. 4B). The initial contraction of the descending distention-induced response of the anal canal was decreased by atropine (3 x 10^{-7} M) while the relaxation was not changed. In the presence of atropine, spantide (10^{-7} M) decreased further the contraction and did not alter the relaxation. L-NNA (5 x 10^{-4} M) increased the contraction and significantly inhibited the relaxation (1.12 ± 0.10 mN, n = 8, $p < 0.05$). L-Arginine (5 x 10^{-4} M) showed an opposite effect to that of L-NNA. L-Arginine decreased the initial contraction and extended the relaxation in the descending distension-induced motor response of the anal canal in atropine-pretreated preparations (Fig. 4C).

Effects of TTX

The addition of TTX at a concentration of 10^{-7} M for 10 min in the oral or in the anal compartment of the bath prevented the electrically- or balloon distension-induced descending motor responses of the anal canal (n = 4, Data not shown).

ChAT-, substance P- and NADPH-diaphorase-positive structures

The immunohistochemical study for ChAT revealed a few positive nerve cell bodies and a large number

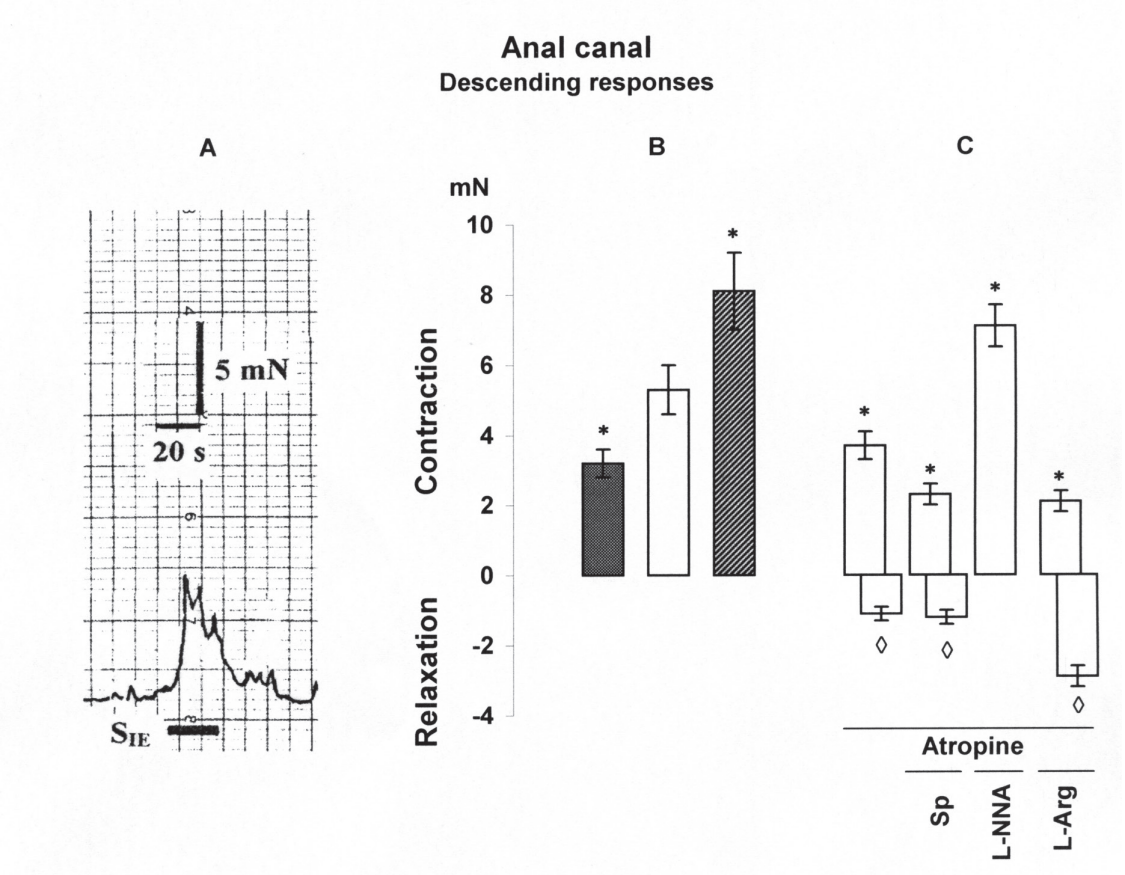

Fig. 3. - Typical mechanographic record showing electrically-elicited descending motor response of anal canal (Sie) in isolated rat recto-anal segment (A); Descending motor responses of anal canal elicited by EFS applied at frequencies of 2 Hz (■), 5 Hz (□) or 10 Hz (▨) (B); Descending motor responses of anal canal elicited by EFS applied at frequency of 5 Hz in the presence of Atropine (3 x 10^{-7} M) or Atropine plus Spantide (Sp, 10^{-7} M) or Atropine plus NG-nitro-L-arginine (L-NNA, 5 x 10^{-4} M) or Atropine plus L-Arginine (5 x 10^{-4} M) (C). The values represent means ± s.e. mean of at least 8 experiments. Symbols indicate: significant differences at $p \leq 0.05$, 't' test ($t \geq 2.31$ at 8 experiments) - (*) drug treatment *vs.* controls and (◊) opposite effects *vs.* controls.

of varicose nerve fibers in the myenteric ganglia of the anal canal (Fig. 5A). Substance P-immunostained varicose nerve fibers formed a rich meshwork around SP-negative nerve cell bodies. Many varicose substance P-immunopositive fibers were also observed in the nerve bundles running between the myenteric ganglia and the muscle layers. Substance P-containing nerve cell bodies were rarely seen there (Fig. 5B). Single immunoreactive nerve fibers ran parallel to the myocytes. The myenteric ganglia in the anal canal were well outlined by many NADPH-diaphorase-reactive nerve cell bodies and their processes (Fig. 5C). Positive neurons display a multipolar shape and the initial parts of their dendrites could be seen as well. In addition, numerous varicose fibers in the ganglia and internodal strands were evident. In the longitudinal muscle layer scattered positive fibers were found. In the circular muscle layer a large number of sparse varicose nerve fibers and thick bundles were dispersed throughout.

Discussion

This study evaluated reflex evacuation of the anal canal with anatomically and functionally intact anal sphincters. The electrically- or distention-induced motor responses of the anal canal in a rat model preparation were neurogenic in nature since they were prevented by TTX, a blocker of neuronal action potentials. The partitioned organ bath method used in the experiments allowed evaluation of the electrically-elicited local or descending motor responses as well as comparison of the different

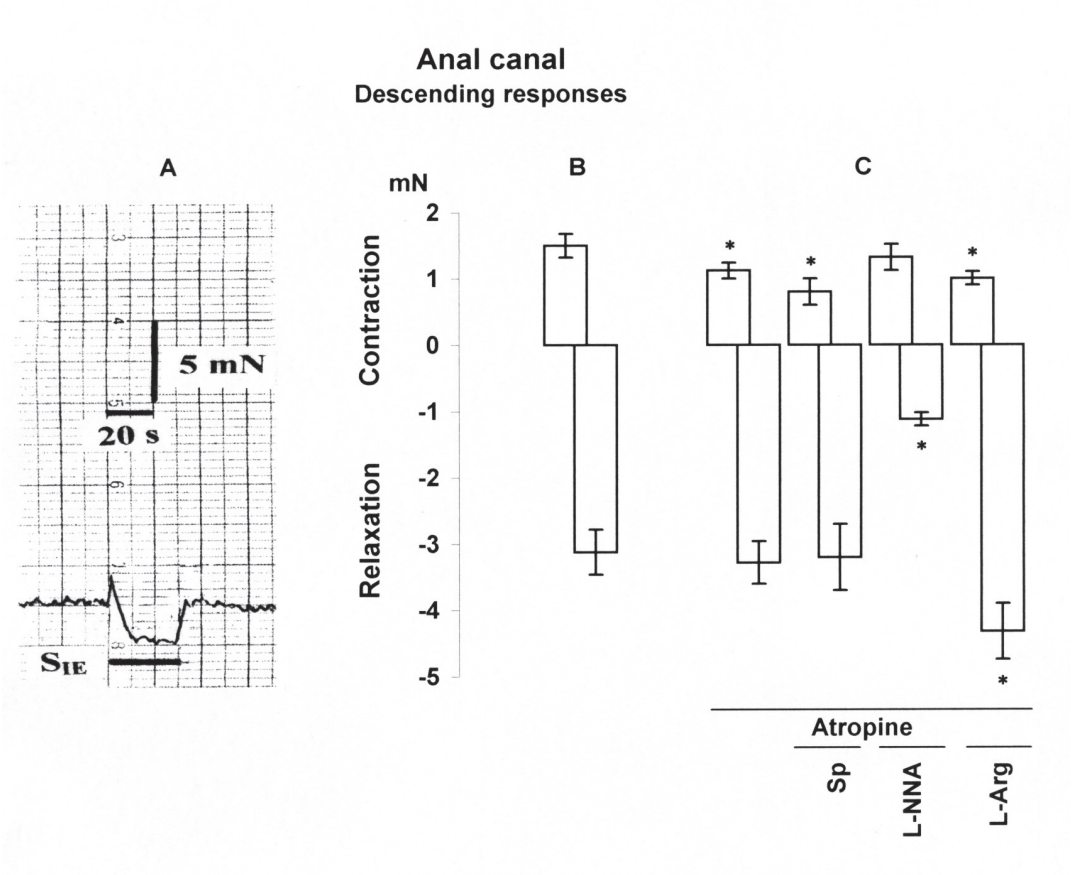

Fig. 4. - Typical mechanographic record showing balloon-inflation induced descending motor response of anal canal (Sie) in isolated rat recto-anal segment (A); Control descending motor response of anal canal induced by balloon-inflation (B); Descending motor responses of anal canal induced by balloon-inflation in the presence of Atropine (3 x 10^{-7} M) or Atropine plus Spantide (Sp, 10^{-7} M) or Atropine plus NG-nitro-L-arginine (L-NNA, 5 x 10^{-4} M) or Atropine plus L-Arginine (5 x 10^{-4} M) (C). The values represent means ± s.e. mean of at least 8 experiments. Symbols indicate: significant differences at $p \leq 0.05$, 't' test ($t \geq 2.31$ at 8 experiments) - (*) drug treatment *vs.* controls.

pattern of descending motor responses of the anal canal induced by electrical stimulation and distension. This led to characterization of the functionally different activation-dependent descending reflex pathways involved in an integrative recto-anal neuronal circuitry.

We observed that electrical stimulation applied to the anal canal or to the distal part of the rectum elicited contractile local or descending responses of the anal canal. The local responses may reflect the activation of neuro-neuronal and/or neuro-muscular communications of the anal canal area while the descending contractions could be considered a result of the activation of nerve pathways along the distal rectum. The effect of electrical stimulation consisted of stimulatory and/or inhibitory components and is the consequence of a propagation of an action potential causing local release of neurotransmitters (Paton and Vizi, 1969; Kadlec et al., 1986). The increase of stimulus frequency enhanced the amplitude of both local and descending contractions without changing the pattern of responses suggesting that the increase of contractile responses was due to the frequency-dependent release of the same neurotransmitters. It is known that electrical stimuli applied at higher frequencies recruit more nerve terminals into the process of release of neurotransmitters release (Kadlec et al., 1986). Our finding that the magnitude of electrically-elicited descending responses of the anal canal was less expressed compared to the 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 (Kadlec et al., 1990). Having in mind that both local and descending motor responses of the anal canal

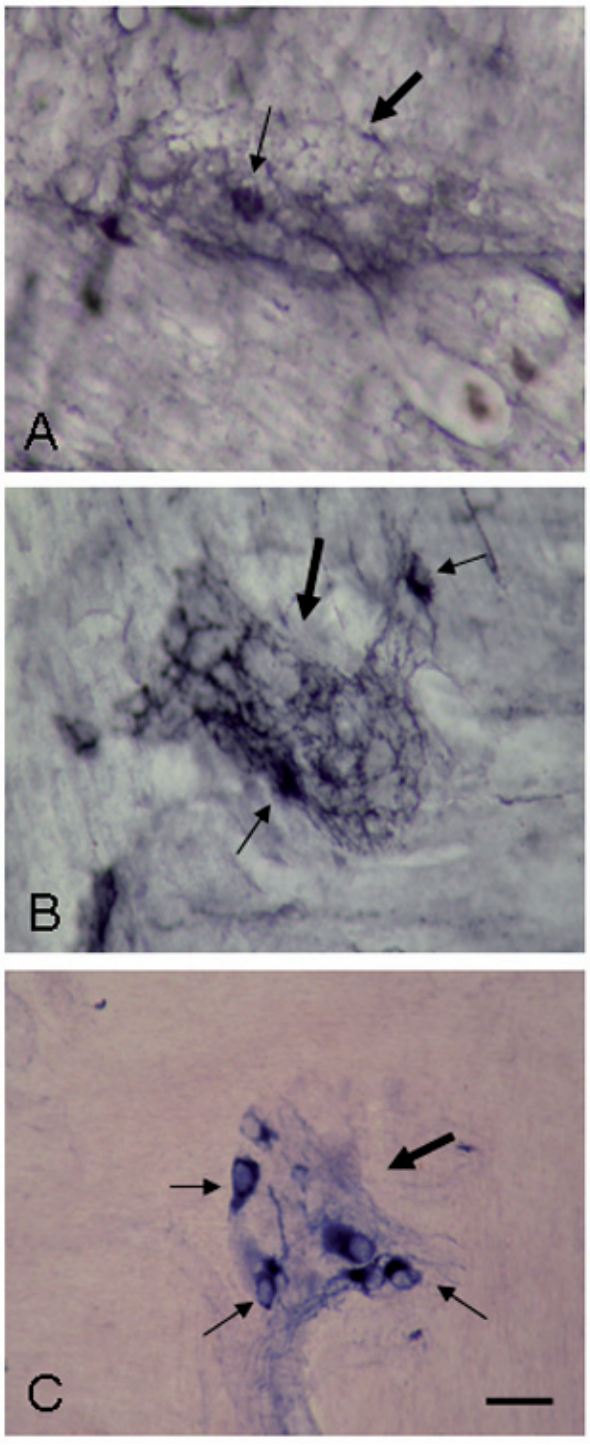

Fig. 5. - Photomicrographs showing the myenteric plexus in the rat anal canal. (A) ChAT-immunoreactivity. The large arrow indicates the myenteric ganglion and the small arrow points to the positive nerve cell body. (B) Substance P-immunoreactivity. In the myenteric ganglion (large arrow) many positive nerve fibers encircled the immunonegative perikarya. The small arrows point to the Substance P-immunopositive perikarya. (C) NADPH-d-staining. The large arrow shows the ganglion and small arrows point to the positive nerve perikarya. Scale bar = 50 μm.

due to electrically-activated nerve structures are contractions it could be speculated that mechanisms of direct excitation of recto-anal nerve structures are involved in the contractile motor events of the anal canal that underlie the storage of rectal content.

The distension-induced descending motor response of the anal canal was opposite in pattern compared to the electrically-elicited descending response. The distension-sensitive descending response was characterized by a short contraction followed by a deep relaxation. The distension of the rectal wall is a factor leading to excitation of mechanoceptors involved in the monitoring of the filling state and the contraction level of human and cat's rectum (Sun et al., 1990; Penninckx et al., 1992; Ruhl et al., 1998). A dense afferent innervation by mechanoceptors with specialized intraganglionic laminal endings detecting mechanical deformation is described in guinea pig rectum (Lynn et al., 2003; Zagorodnyuk et al., 2005; Lynn et al., 2005). Rectal wall stretch induced by rectal content is sufficient to initiate the defecation reflex in humans (Shafik et al., 2006). Obviously, the activation of mechanoceptor-dependent descending pathways located in the region of distal rectum could be responsible for the relaxation of the anal canal thus providing the propulsion of rectal content.

The present experiments showed that in the recto-anal region of a rat model preparation the pattern of descending reflex motor responses of the anal canal depended on the type of activation of descending reflex pathways that organize a network which could be regarded as a functional module (Costa et al., 2000). Enteric excitatory and inhibitory neurotransmissions act during the evacuation process and underlie contraction/relaxation events (Bharucha, 2006; Kijima et al., 2006). The prevalence of effects of excitatory or inhibitory neurotransmissions closing up to the anal canal and the properties of the defecation reflex in rat has been scarcely investigated (Nagano et al., 2004). Recently it was indicated that cholinergic and excitatory non-cholinergic neurotransmitters caused contractile responses of the rat anal canal (Radomirov et al., 2009a).

We observed that the descending electrically-elicited contractile responses or the contractile components of the distension-induced responses of the anal canal were significantly decreased but not prevented by atropine. Since in the presence of atropine a relaxation was observed in the electrically-elicited

responses and the relaxation of the descending distension-induced responses increased by magnitude it could be suggested that inactivation of the effect of excitatory cholinergic neurotransmission unmasked an action of inhibitory neurotransmission(s). Nitric oxide released from non-adrenergic non-cholinergic nerves is proposed as an inhibitory agent in the recto-anal region (Rand and Li, 1995; Stebbing et al., 1996; Cook et al., 2001). The present results demonstrated that nitric oxide-related drugs affected the descending electrically- or distension-induced motority of the anal canal. L-NNA, an inhibitor of nitric oxide synthase, increased the contractions or the contractile components of the responses during atropine treatment and decreased the relaxation while L-Arginine, a substrate of nitric oxide synthesis, decreased the contractile activity and increased the relaxation. These observations are in concert with the view that nitric oxide could modulate transmitter release from motor nerve endings. The L-NNA-provoked blocking of nitric oxide synthesis prevents the direct inhibitory action of nitric oxide and leads to suppression of the negative modulation on acetylcholine release (Bartho and Lefebvre, 1995; Smith and McCarron, 1998; Timoteo et al., 2008). In the guinea pig recto-anal region L-NNA enhances the reflex-mediated contraction of the rectum and abolishes the reflex-mediated relaxation of the internal anal sphincter (Yamanouchi et al., 2002). We observed that in the rat recto-anal region the block of cholinergic receptors allowed manifestation of excitatory neurotransmitter(s) except the cholinergic one. Spantide, a NK-1 tachykinin receptor antagonist lowered further the atropine-provoked decrease of contractions suggesting the involvement of Substance P in contractile events. Recent data propose substance P as an excitatory mediator of the internal anal sphincter (Yang et al., 2006). Thus, the increase of contractile components in responses of the rat anal canal due to treatment with L-NNA in the presence of atropine could be attributed to release of substance P. Nitric oxide-dependent substance P-mediated neurotransmission is described in guinea pig small intestine (Wiklund et al., 1993). L-Arginine, acting similarly to nitric oxide donors (Ballester et al., 2007) decreased the contractions and increased the relaxation in the descending responses confirming the role of nitric oxide in the neural pathways in the rat recto-anal region.

The nitric oxide-related drugs affected both contraction and relaxation characterizing the electrically- or distension-induced descending reflex motor responses of the anal canal indicating that nitric oxide is an important contributor to the function of both excitatory and inhibitory descending reflex pathways in the neuronal modular network of the rat recto-anal region. The effects of nitric oxide were assessed as a new form of interneuronal interactions (Kiss and Vizi, 2001) and according to Vizi (2000) nitric oxide is an ideal mediator taking part in mechanisms of nonsynaptic communications. Such universal action of nitric oxide most probably has a role in the modulation of rat recto-anal evacuation motor activity. However, we observed that the contractile and relaxant activity of descending electrically-elicited or distension-induced responses of the anal canal occurred in the presence of nitric-oxide related drugs which indicates that other nervous pathways, probably excitatory adrenergic and inhibitory peptidergic pathways may play a role in the modulation of the anal canal motility (Matsufuji and Yokoyama, 2003; Rattan, 2005; Park et al., 2007). Our histochemical and immunohistochemical results on the expression of NADPH-diaphorase-, ChAT- and substance P-positive nerve structures in the myenteric ganglia and muscle layers of the anal canal suggest an important role of acetylcholine, substance P and nitric oxide in the transmitter pathways and implicate these transmitters in the recto-anal motor activity.

In summary, this study evaluated electrically- or distension-induced descending reflex motor responses of the anal canal in a rat model preparation. The motor responses of anal canal depended on the type of activation applied to the descending reflex pathways in the distal part of rectum. Electrical stimulation displayed contractile responses of the anal canal. The distension of the rectal wall manifested motor responses consisting of a short contraction followed by a long-lasting deep relaxation. The difference in the pattern of responses suggests involvement of neurotransmission(s) acting during different phases of the evacuation process. Most probably cholinergic and tachykininergic neurotransmissions are involved in the contractile events of the anal canal that underlie the storage of rectal content while mechanoceptor-sensitive nitrergic-dependent pathways are responsible for the relaxation of the anal canal thus providing for the propulsion of rectal content.

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