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Effect of acetic acid or trypsin application on rat colonic motility in vitro and modulation by two synthetic fragments of chromogranin A

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Abstract

The hypothesis that Chromogranin A (CgA)-derived peptides are involved in mechanisms modulating altered colonic motility was tested. Rat distal colonic strips were studied using an organ bath technique. Acetic acid (AA)-induced effects were characterized on spontaneous mechanical activities (SMA) in the presence of CgA4–16 or CgA47–66. In preparations with mucosa, AA induced a transient hyperactivity followed by a decrease in tone. The first phase is sensitive to tetrodotoxin (TTX) and capsaicin. The second phase was sensitive to BAYK8644 but insensitive to L-nitro-arginine-methyl-ester (L-Name)/apamin together. CgA4–16 or CgA47–66 alone produced no change on SMA. The administration of CgA4–16 prior to AA increased the duration of the excitatory component and reduced tone inhibition. CgA47–66 prior to AA only decreased duration of the excitatory phase. In preparations without mucosa, AA decreased tone. This effect was sensitive to BAYK8644 and CgA4–16. Trypsin decreased basal tone. This effect was suppressed by TTX, BAYK8644 or L-Name/apamin and were reduced by CgA4–16. AA-induced effects on rat colonic motility in vitro may be mediated through activation of primary afferents and an action at L-Type calcium channels. CgA-derived peptides are shown to decrease AA-induced effects on motility.

Keywords: Calcium; Chromogranin; Colon; Enteric nervous system; In vitro; Motility

1. Introduction

Very little is known about a potential functional role of Chromogranin A (CgA) in gastrointestinal physiopathology, although CgA seems to be overexpressed within endocrine cells from Crohn disease patients [1]. Furthermore, one study mentioned that immunoreactivity for CgA is also localized within the enteric nervous system [2]. Vasostatin, corresponding to the sequence within the first 76 residues of CgA, displays vasoinhibitory activities [3,4], exerts negative inotropism in the working heart activities [5] and modulates calmodulin action [6–8]. CgA 4–16 and 47–66 are two peptides derived from vasostatin. CgA4–16 exerts negative inotropism in the working heart activities from frog [9]. CgA47–66 was shown to display antimicrobial and fungal activities [10]. Irritable bowel syndrome (IBS) is characterized by prominent alteration of muscle contractions [11]. It has been hypothesized that altered peripheral function of visceral afferents and the central processing of afferent information might be important factors in the altered visceral sensitivity observed in IBS patients. Previous in vivo studies have shown that CgA 4–16 and 47–66 peptides modulate peripheral nociception [12,13]. Moreover, it has been shown that inflammation reduced resting membrane potential and both amplitude and duration of slow waves in circular muscle cells [14]; it also decreased muscle tone. Acetic acid (AA) is commonly used to induce acute colonic inflammation and

Abbreviations: AA, acetic Acid; CgA4–16, Chromogranin A 4–16 peptide; CgA47–66, Chromogranin A 47–66 peptide; CGRP, calcitonin gene related peptide; L-Name, L-nitro-arginine-methyl-ester; SMA, spontaneous mechanical activities; SP, substance P; TTX, tetrodotoxin.

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pain in vivo. Injury can result in inflammatory processes that decrease pH [15]. AA is associated with a decrease in pH that has been shown to activate nociceptors [16]. We have previously shown that CgA4–16 increased the nociceptive effect induced by intraperitoneal AA [12] and CgA47–66 displayed significant antinociceptive effects using the same in vivo model [13].

Therefore, the aim of the present work was to investigate the effects of AA on rat smooth muscle contractions in vitro and to determine the possible sites of action of CgA4–16 and CgA47–66 on AA-induced effects, using an in vitro organ bath technique associated to pharmacological techniques. To precise the possible site of action of CgA-derived peptides, preparations with and without mucosa were studied.

2. Materials and methods

2.1. Animals and technique

Male Wistar rats (250–500 g) fasted 24 h before experiments with free-access to water were used in the present study. Animals were anaesthetized with urethane 20% (1 ml/100g). After a midline laparotomy, distal colon was removed and placed in a Petri dish containing a physiological Krebs solution (in mM: 119.9 NaCl, 6 KCl, 15.6 $NaHCO_3$, 1.2 $MgCl_26H_2O$, 11.7 Glucose, 2.5 $CaCl_2$, $2H_2O$, pH 7.4). Rats were killed by a overdose of anesthetic. The segment of distal colon was rinsed of intraluminal content. Annular smooth muscle pieces were mounted in organ bath chambers containing 4 ml of warmed (37 °C) and gassed (95% O_2, 5% CO_2) Krebs solution. In a second set of experiment, mucosa-free circular smooth muscle strips were dissected from another group of rats. Circular smooth muscle activities were measured by means of an isometric force transducer (PowerLabs8/sp, ADInstruments, USA) and amplifier (Powerlabs, ADInstruments) and were visualized using personal computer (HP Brio 410, Hewlett-Packard, USA).

All animal handling and experimental procedures were approved by the local Animal Care and Ethic Committees.

2.2. Preparation and analysis of synthetic peptides

Human CgA4–16 (NSPMNKGDTEVMK) and Human CgA47–66 (RILSILRHQNLLKELQDLAL) were synthesized in our laboratory on an Applied Biosystems 432A peptide synthesizer SYNERGY (Foster City, USA), using the stepwise solid-phase synthetic approach with 9-fluoromethoxycarbonyl (Fmoc chemistry) [17]. Then, the synthetic peptides were purified by chromatography on a C18 column (Vydac, Hesperia, USA; 218 TP 10×250 mm). After lyophilization, synthetic peptides were analyzed by sequencing and mass spectrometry. The sequences of synthetic CgA4–16 and CgA47–66 were determined in our laboratory by automatic Edman degradation on an Applied Biosystems 473A sequencer (Applied Biosystems/Perkin Elmer, Boston, USA) at the picomolar range. Samples purified by HPLC were loaded on polybrene-treated and precycled glass-fiber filters [18]. Phenylthiohydantoin-amino acids were identified by chromatography on a C18 column (PTH C18, 2.1×200 mm). The analysis by mass spectrometry was performed using Matrix-Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (BIFLEX™ Bruker, Wissembourg, France), according to the procedure previously reported [19]. Preserving of molecular integrity of CgA4–16 and CgA47–66 in the presence of AA has been controlled using HPLC technique.

2.3. Compounds

The following drugs were used and abbreviations as well as respective suppliers given in parentheses: nifedipine, BAYK8644, capsaicin, L-nitro-arginine-methyl-ester (L-Name), apamin, tryspin (from porcine pancreas), human calcitonin gene-related peptide (human CGRP) (Sigma Aldrich L'Isle d'Abeau Chesne, France); tetrodotoxin (TTX) (Tocris, Illkirch, France); and acetic acid (AA) (Merck, Illkirch, France).

All substances, excepted CGRP, BAYK8644, and nifedipine were dissolved in distilled water. CGRP was dissolved in 0.1% BSA (Sigma Aldrich). BAYK8644 was dissolved in 0.1 N NaOH and nifedipine in absolute ethanol. Solvent vehicles were added to the bath (in a volume of 4 μl in 4 ml) for control experiments. They showed no effect on spontaneous mechanical activities (SMA) developed by circular muscle strips from rat distal colon.

2.4. Experimental design

At the beginning of each experiment, strips were stretched to their optimal resting tension: this was achieved by step-wise increases in tension (+5 mN) until tension reached an stable amplitude of 16 mN . Strips were allowed to equilibrate for 60 min whereupon the effects of various pharmacological agents were investigated. Each addition of drug was followed by a period of time necessary to reach the maximal effect.

In a first series of experiments, AA (0.01%; 0.05% or 0.1%) was added to the bath and the pharmacological effects were characterized. The pH in the bath decreased respectively from 7.4 to 7.2±0.2, 6.8±0.1 and 6.5±0.3. In a second series of experiments, CgA4–16 or CgA 47–66 fragments were added 10 min prior to AA (0.05%) administration. In the presence of CgA-derived peptides, the pH did not change compared to AA at different doses alone. After each experiment, tissues were washed out by replacing the bath solution several times with fresh Krebs solution in order to recover control SMA. If this latter condition was not fulfilled, the muscle strip was not used for further experiments. Concentration response curves to AA