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Relaxation induced by N-terminal fragments of chromogranin A in mouse gastric preparations

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Abstract

A definitive role for chromogranin A (CGA)-derived fragments in the control of the gastrointestinal smooth muscle contractility has not been yet established. The purpose of the present study was to evaluate, *in vitro*, the effects of the recombinant vasostatin 1–78 (VS-1), CGA 7–57 and CGA 47–66 on the mouse gastric mechanical activity, recording the changes of intraluminal pressure. VS-1, CGA 7–57 and CGA 47–66 produced concentration-dependent relaxations. Mouse anti-vasostatin-1 monoclonal antibody 5A8, recognising the region 53–57, abolished the relaxation induced by VS-1, indicating the specificity of the effect. The relaxation was significantly reduced by tetrodotoxin (TTX), blocker of neuronal voltage-dependent Na^+ channels, L-NAME, inhibitor of nitric oxide (NO) synthase, or apamin, blocker of small conductance Ca^{2+}-dependent K^+ channels. The joint application of TTX and L-NAME did not show any additive effects, whereas TTX plus apamin abolished the VS-1 response. The results suggest that the N-terminal CGA-derived peptides are able to relax mouse gastric muscle and, therefore, they point out an inhibitory role of vasostatin I in the gastrointestinal tract. The relaxation is mediated in part by neural mechanisms through NO production and in part by non-neural mechanisms involving the opening of small conductance Ca^{2+}-dependent K^+ channels.
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1. Introduction

Chromogranin A (CGA) belongs to a family of acidic-secretory proteins, co-stored and co-released with neurotransmitters and hormones in the diffuse neuroendocrine system, including the chromaffin cells of the gastrointestinal tract [1–3]. CGA has been postulated to be a prohormone, because, in many tissues after its release, it is rapidly processed by pro-hormone convertases and other proteases in a tissue- and species-specific manner, giving rise to several regulatory peptides, which exert a multitude of biological activities [4,5]. Among these peptides, vasostatin I (CGA 1–76) is one of the predominant product and it shows, together with the peptides derived from own cleavage (CGA 7–57; CGA 47–66, etc.), various biological effects. They include: inhibition of fungal growth [6], proadhesive effects on human fibroblasts and vascular smooth muscle cells [7], vasoinhibitor and cardiosuppressive activities [8–14].

Very little is known about the possible roles of CGA and its derived-peptides fragments in gastrointestinal physiopathology, although there are evidences of an CGA-overexpression in enterochromaffin cells in some colonic pathology, as Crohn's disease [15]. Recent researches have shown that the vasostatin I-derived fragments, such as CGA4–16 or CGA 47–66, may modulate the contractility of human or rat colon in condition reproducing the inflammatory state [16,17], suggesting the hypothesis about a preventive role for vasostatin I-derived peptides in the alteration of colonic motility induced by inflammation. In addition, our group have shown that the recombinant human CGA N-terminal fragment Ser-Thr-Ala-CGA 1–78 (VS-1), containing the vasostatin I (CGA 1–76) and the synthetic peptide, CGA 7–57, plays an inhibitory modulatory role on the spontaneous mechanical activity of rat proximal colon, through an apamin-sensitive mechanism

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involving, at least in part, a neural nitrergic pathway [18]. To date, researches have been just performed using human or rat colon, while nothing is known about the mouse gastrointestinal tract, although this animal is frequently used for knocking out the expression of messenger molecules in studies directed to determine their physiological role. Thus, information on the putative modulatory role of CGA-derived peptides in the normal mouse is an essential prerequisite for such an approach, also in consideration that CGA immunoreactive cells have been demonstrated throughout the mouse gastrointestinal tract [19] with the highest frequency in the upper regions (stomach and duodenum) [20].

Therefore, the purpose of the present study was to evaluate the influence of VS-1 on the mouse gastric spontaneous mechanical activity using the isolated whole organ, in order to analyse the muscle function under conditions where the influence of external factors is removed, but the muscle itself performs in a manner analogous to its in vivo capacity. In particular, the responses induced by the recombinant peptide VS-1 and by CGA 7–57 and CGA 47–66 synthetic peptides were investigated and the mechanism of action responsible for the observed effects was also examined. We used the human recombinant VS-1 which corresponds to the sequence CGA 1–78 bearing the tripeptide Ser-Thr-Ala-at the N-terminal end. The N-terminal sequence of mouse CGA presents a high structural homology, which reaches 100% in CGA 45–75 [21]. On the other hand, the human recombinant peptide has been previously shown to produce inhibitory effects on the rat proximal colon [18].

2. Materials and methods

Experiments, authorized by the Ministero della Sanità (Rome, Italy), were performed on adult mice (C57BL/10SnJ), killed by cervical dislocation. The abdomen was immediately opened, the esophagus was tied proximal to lower esophageal sphincter, and the entire stomach was excised. Preparations were mounted in a custom designed organ bath, which was continuously perfused with oxygenated (95% O_2 and 5% CO_2) and heated (37 °C) Krebs solution with the following composition (mM): NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2; $CaCl_2$ 2.5; glucose 11.1. The pyloric end was tied around the mouth of a J-tube, which was connected to a standard pressure transducer (Statham Mod. P23XL; Grass Medical Instruments, Quincy, MA, USA). The changes of endoluminal pressure were recorded on ink-writer polygraph (Grass model 7D). Preparations were allowed to equilibrate for about 60 min before starting the experiment.

2.1. Experimental protocol

At the beginning of each experiment, to establish the sensitivity of the preparation, this was challenged with isoproterenol (1 μM) until reproducible responses were obtained. Isoproterenol was added into the bath after switching off the perfusion and left in contact with the preparation for 2 min. The responses to non-cumulative concentrations of VS-1 (1 nM–0.3 μM), CGA 7–57 (10 nM–1 μM) and CGA 47–66 (10 nM–1 μM) were examined on the gastric basal tone. The substances were added into the bath at increasing concentrations in volumes of 50 μl. Each concentration was left in contact with the tissue for 7 min. In some experiments, to confirm the specificity of the observed effect, VS-1 (3 nM) was tested after mixing it with mouse anti-CGA monoclonal antibody (mAb) 5A8 (0.1 μM) recognizing the region 53–57 [7]. The response to VS-1 was also tested in presence of tetrodotoxin (TTX) (1 μM), a voltage-dependent Na^+-channel blocker, N_ω-nitro-L-arginine methyl ester (L-NAME) (300 μM), an inhibitor of nitric oxide (NO) synthase, and apamin (0.1 μM), a blocker of small conductance Ca^{2+}-dependent potassium channels. These agents were added to the perfusing solution at least 30 min before testing the CGA-derived peptides. Previously we showed that the concentrations used and the exposure time to these blocking drugs were appropriate to achieve maximal effects [22,23].

2.2. Data analysis and statistical tests

Relaxant responses to CGA-derived peptides were expressed as a percentage of the response produced by isoproterenol (ISO, 1 μM). The concentration (EC_{50}) with 95% confidence intervals (CIs) producing half maximum response was calculated using Prism 4.0, GraphPad (San Diego, CA, USA). All data are expressed as mean values±SEM. The letter *n* indicates the number of experiments and it is equivalent to the number of experimental animals. Statistical analysis was performed by

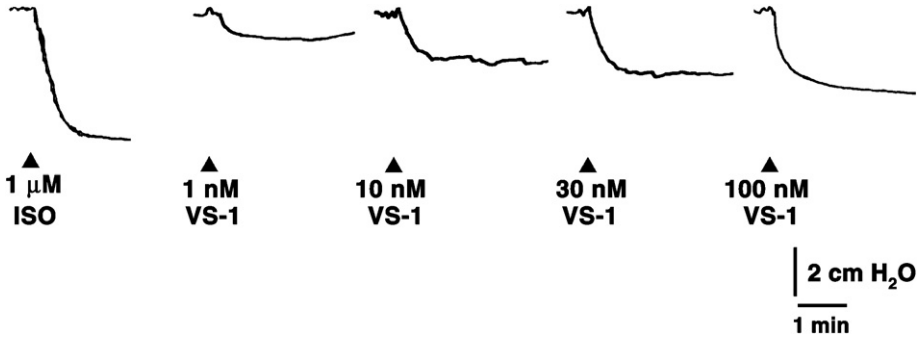

Fig. 1. Original tracings illustrating the effect of isoproterenol (ISO; 1 μM) and VS-1 (1–100 nM) on mouse gastric preparation. The arrows indicate the time of drug addition.

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