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Cytoskeleton mediates negative inotropism and lusitropism of chromogranin A-derived peptides (human vasostatin1-78 and rat CgA_{1-64}) in the rat heart

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ABSTRACT

Cytoskeleton scaffold in cardiac myocytes provides structural support and compartmentalization of intracellular components. It is implicated in cardiac pathologies including hypertrophy and failure, playing a key role in the determinism of contractile and diastolic dysfunctions. Chromogranin A (CgA) and its derived peptides have revealed themselves as novel cardiovascular modulators. In humans, normal CgA levels considerably increase in several pathologies, including heart failure. Recent data have shown on the unstimulated rat heart that human recombinant Vasostatin-1 (hrVS-1) and rat chromogranin A 1-64 ($rCgA_{1-64}$) induce negative inotropic and lusitropic effects counteracting the β-adrenergic-dependent positive inotropism with a functional non-competitive antagonism. This study investigates, on the isolated Langendorff perfused rat heart, whether cardiac cytoskeleton is involved in the modulation of contractility and relaxation exerted by hrVS-1 and $rCgA_{1-64}$. Cytoskeleton impairment by either cytochalasin-D (actin polymerization inhibitor), BDM (myosin ATP-ase antagonist) or wortmannin (inhibitor of PI3-K/Akt transduction cascade), or W-7 (calcium–calmodulin antagonist) abolished hrVS-1 and $rCgA_{1-64}$-mediated inotropism and lusitropism. Using fluorescent phalloidin, we showed on rat cardiac H9C2 cells that hrVS-1 (10 nM ÷ 10 μM) stimulates actin polymerization. Taken together these data indicate that in the rat heart, the actin cytoskeletal network strongly contributes to the cardiotropic action of CgA-derived peptides.

1. Introduction

Chromogranin A (CgA) is an acidic protein co-stored with catecholamines (CAs), nucleotides, calcium and other peptide hormones in the secretory granules of several endocrine and neuronal cells and is released in the extracellular environment by exocytosis [1]. In the rat heart, CgA immunolocalizes in myocardiocyte secretory vesicles together with atrial natriuretic peptide (ANP) [2]. It was also immunodetected in the human ventricular myocardium in co-localization with brain natriuretic peptide (BNP) [3].

Apart from an intracellular role in secretory vesicle biogenesis [4], CgA exerts an important extracellular function as a prohormone leading to the formation of different shorter biologically active peptides produced *via* a tissue-specific proteolytic processing, possibly also at cardiac level (e.g. CgA_{4-113}, CgA_{1-124}, CgA_{1-135} and CgA_{1-199}; [5]). In humans, CgA and its derived fragments are released in the blood in response to stress, reaching nanomolar concentrations in the peripheral circulation [1]. Thus, they appear to function as endocrine/paracrine cardiac stabilizers, particularly under intense adrenergic stimuli (*e.g.* stress responses: [6]). In the presence of neuroendocrine tumors [7,8], renal and liver failure [9], these levels significantly increase. Notably, patients with chronic heart failure and complicated myocardial infarction show higher CgA concentrations which correlate with the severity of the disease, representing a prognostic indicator of mortality [10,11]. The evidence that CgA-knockout mice show hypertension and cardiac enlargement [12], together with CgA and BNP coordinated over-expression in dilated and hypertrophic cardiomyopathy [3], suggests a cardiac neuroendocrine

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protective role for CgA and its fragments and a potential diagnostic and therapeutic applications in heart failure.

Increasing physio-pharmacological evidence in both mammalian and non-mammalian vertebrates, indicate that peptides containing the N-terminal domain of CgA (e.g. human recombinant $CgA_{1\text{-}78}$, $CgA_{7\text{-}57}$, $CgA_{1\text{-}76}$) contribute to the autocrine/paracrine cardiac regulation. Their major actions consist of both negative inotropism (eel, frog, and rat) and lusitropism (rat), and in the counteraction of the β-adrenergic-mediated positive inotropism, typically induced by isoproterenol (Iso) on eel, frog and rat [3,6,13,14]. Moreover, preconditioning with human recombinant $CgA_{1\text{-}78}$ in the rat heart protect against ischemia/reperfusion injury [15].

Recently, the synthetic rat CgA sequence ($rCgA_{1\text{-}64}$) accounts for the highly conserved domain of the human vasostatin-1 ($CgA_{1\text{-}76}$), corresponding to human N-terminal CgA-derived vasostatin-1 (VS-1), was found to induce negative inotropism and lusitropism, as well as coronary dilation on the Langendorff perfused rat heart. It also counteracts Iso- and ET-1-induced positive inotropic effects and ET-1-dependent coronary constriction [16] and depresses basal and Iso-induced contractility on rat papillary muscles, without affecting calcium transients on isolated ventricular cells [16] with non-competitive antagonistic action indirectly via endothelium-derived NO [15,16].

The cytoskeleton is a flexible and dynamic structure involved in a variety of essential cellular functions including motility, maintenance of cell shape, cell attachment/interaction with the extracellular matrix (ECM) and anchorage of cell organelles [17]. It contributes to the modulation of several ion currents [17], intracellular trafficking, organelle transport within the cell, and in different steps of endocytosis [18,19]. In human and bovine endothelial cells, $CgA_{1\text{-}76}$ is able to prevent cytoskeletal reorganization induced by TNFα, VEGF, thrombin and pertussis toxin [20,21]. As shown by functional experiments on eel and frog hearts [23], cytoskeleton is under the influence of CgA-derived fragments.

We report here in the rat that the cardio-suppressive effects induced by either human recombinant STA-$CgA_{1\text{-}78}$ (hrVS-1) and $rCgA_{1\text{-}64}$ are blocked by the inhibition of cytoskeletal dynamics induced by cytochalasin-D, wortmannin, butanedione 2-monoxime (BDM) and N-(6-aminohexil)-5-chloro-1-naphthalenesulfonamide (W7). This suggests the cytoskeleton as an important determinant of the signal-transduction cascade which underlies the inhibitory influence exerted by CgA-derived peptides on the contractile myocardial machinery.

2. Materials and methods

2.1. Animals

We used male Wistar rats weighing 220–240 g (Harlan Italy Srl, Udine, Italy). Animal care, sacrifice and experiments were supervised according to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

2.2. Isolated Langendorff heart preparation

Hearts were rapidly excised, transferred in ice-cold buffered Krebs-Henseleit solution (KHs) after the rats have been anesthetized with intraperitoneal injection of ethyl carbamate (2 g/kg rat, i.p.). As previously described [24], aorta was immediately cannulated with a glass cannula and connected to the Langendorff apparatus to start perfusion at constant flow-rate (12 ml/min/g wet weight of the heart). Briefly, the apex of the left ventricle (LV) was pierced to avoid fluid accumulation. A water-filled latex balloon, connected to a BLPR gauge (WPI, Inc. Sarasota, FL, USA), was inserted through the mitral valve into the LV to allow isovolumic contractions and to continuously record mechanical parameters. The balloon was progressively filled with water to obtain an initial left ventricular end diastolic pressure of 5–8 mm Hg. Another pressure transducer located just above the aorta recorded coronary pressure (CP). Haemodynamic parameters were assessed using a PowerLab data acquisition system and analyzed using Chart software (both purchased by ADInstruments, Basile, Milano, Italy).

2.3. Drugs and chemicals

Human Recombinant STA-$CgA_{1\text{-}78}$ (hrVS-1) was produced and characterized as previously described [25]. Rat $CgA_{1\text{-}64}$ ($rCgA_{1\text{-}64}$) was synthesized in "Inserm U575 Physiopathology of Nervous System", Strasbourg-France on an ABI 431 A (Applied Biosystems, Inc., Foster City, CA, USA) peptide synthesizer using the standard procedures of FMOC (9-fluorenylmethoxycarbonyl) chemistry [26]. Cytochalasin-D, DMEM, antibiotics (penicillin and streptomycin, phalloidin-TRIC and Hoechst 33258 were purchased from Sigma Chemical Company; St. Louis, MO, USA). BDM, wortmannin and W7 were purchased from Calbiochem (Milan, Italy). Cytochalasin-D, wortmannin and W7 were used in a darkened perfusion apparatus to prevent degradation. All stock solutions were prepared in double-distilled water; in particular, cytochalasin-D was primarily dissolved in small amounts of ethanol (0.1 mg/ml) and BDM (10 mg/ml), W7 (5 mg/ml) and wortmannin (25 mg/ml) in DMSO. Stock solutions (10^{-4} M) of each drug were obtained by adding double-distilled water; dilutions were made in Krebs solution immediately before use.

Preliminary experiments showed that the presence of equivalent amounts of ethanol and DMSO in Krebs without drugs did not modify basal cardiac performance.

2.4. Experimental protocols

2.4.1. Basal conditions

2.4.1.1. Langendorff perfused heart. In order to assess the effects of the peptides on cardiac function the well-characterized Langendorff perfused rat heart model was used. Basal parameters of cardiac preparation are shown in Table 1. Endurance and stability of the preparations, analyzed by measuring the performance variables every 10 min, showed that the heart is stable up to 180 min.

Heart performance was evaluated from the LV pressure (LVP, in mm Hg) which is an index of contractile activity, the rate-pressure product (RPP:HR × LVP, in 10^4 mm Hg × beats/min) which is an index of cardiac work, the maximal value of the first derivative of LVP (mm Hg/s) which is an index of the maximal rate of LV contraction, the time to peak tension of isometric twitch (Ttp) which is an assessment of

Table 1
Hemodynamic parameters at basal conditions.

LVP (mm Hg)	89 ± 3
HR (beats min^{-1})	280 ± 7
EDVP (mm Hg)	5–8
RPP (mm Hg beats min^{-1})	$2.5 \pm 0.1 \times 10^4$
+(LVdP/dt)max (mm Hg s^{-1})	2492 ± 129
−(LVdP/dt)max (mm Hg s^{-1})	−1663 ± 70
Time to peak (s)	0.08 ± 0.01
HTR (s)	0.05 ± 0.01
T/−t (mm Hg s^{-1})	1.498 ± 1.84
CP (mm Hg)	63 ± 3

LVP, left ventricular pressure; HR, heart rate; RPP, rate–pressure product; +(LVdP/dt)max, maximal rate of left ventricular contraction; −(LVdP/dt)max, maximal rate of left ventricular pressure decline; time to peak tension of isometric twitch; HTR, half time relaxation; T/−t, the ratio obtained by +(LVdP/dt)max/−(LVdP/dt)max; CP, coronary pressure.
Heart weight (g): 1.2 ± 0.2; left ventricle weight (g): 0.7 ± 0.05; animal weight (g): 230 ± 10; perfusion pressure (mm Hg) 100.