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βγ-CAT, a non-lens βγ-crystallin and trefoil factor complex from amphibian skin secretions, caused endothelium-dependent myocardial depression in isolated rabbit hearts

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ABSTRACT

Previous *in vivo* study demonstrated that βγ-CAT, a newly identified non-lens βγ-crystallin and trefoil factor complex from frog *Bombina maxima* skin secretions, possessed potent lethal toxicity on mammals resulted from hypotension and cardiorespiratory arrest. However, the mechanism of cardiac dysfunction induced by the protein is unknown. Here, we report that βγ-CAT, with dosages of 0.8–3.0 nM, elicited an acute negative inotropic effect in isolated rabbit heart Langendorff preparations, which mimicked acute heart failure. In addition, the effect of βγ-CAT on the hearts was mediated by endothelium-dependent coronary vasoconstriction ($P < 0.01$, compared between endothelium-intact and removal hearts). After βγ-CAT (3.0 nM) treatment, the positive signal of tumor necrosis factor-α (TNF-α) was detected mainly around the endothelial cell layer as detected by *in situ* indirect immunofluorescence, indicating that the release of TNF-α occurred. At the same time, a rapid TNF-α release was detected in primary cultured rabbit endocardial endothelial cells (REECs) treated with βγ-CAT. After addition of βγ-CAT (3.0 nM) for 10 min and 30 min, the TNF-α levels were increased to 57.33 ± 3.22 pg/ml and 60.00 ± 5.35 pg/ml ($P < 0.05$, compared with the control values of 21.67 ± 3.45 pg/ml and 33.70 ± 6.24 pg/ml, respectively). At high concentrations, βγ-CAT interfered with the cell viability of REECs (CC_{50} about 25 nM). Taken together, βγ-CAT was able to induce acute myocardial depression and the toxic effect might be partially explained by the release of TNF-α. The finding provides new information to understand the patho-physiological roles of non-lens βγ-crystallins and trefoil factors.

1. Introduction

Crystallins are structural proteins that define the refractive index and the optical properties of the lens tissue. α-Crystallins are related to the ubiquitous small heat-shock proteins, while βγ-crystallins belong to the same superfamily (Wistow and Piatigorsky, 1988). Ep37 proteins found in embryonic epidermis, cutaneous glands and gastric epithelial cells of amphibian *Cynops pyrrhogaster* (Takabatake et al., 1992; Ogawa et al., 1998), and mammalian Absent In Melanoma 1 (AIM1) mRNAs with different

Abbreviations: βγ-CAT, non-lens βγ-crystallin and trefoil factor complex; TFF, trefoil factor; AIM1, absent in melanoma 1; REECs, rabbit endocardial endothelial cells; CF, coronary flow; CPP, coronary perfusion pressure; LVDP, Left ventricular developed pressure; dP/dt_{max}, maximum rate of left ventricular pressure rise; LDH, lactate dehydrogenase; CK-MB, creatine kinase-MB; PI, propidium iodide.

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transcriptional sizes that are temporally regulated during embryogenesis and also found in adult skin, heart, lung and liver, are non-lens βγ-crystallins described in vertebrates (Ray et al., 1997). So far, little is known about the cellular functions and action mechanisms of these non-lens βγ-crystallins.

The trefoil factors (TFFs) are secreted proteins that are characterized by a conserved motif that consists of some 40 amino acid residues known as the trefoil domain (or P-domain previously) (Sands and Podolsky, 1996; Thim, 1997). One of the main functions of these peptides is in mucosal protection and healing by stimulating the migration of cells at the mucosal wounding edges (Mashimo et al., 1996; Taupin and Podolsky, 2003). TFFs have been shown to interfere with crucial biological processes such as cell proliferation, differentiation, apoptosis and angiogenesis (Taupin and Podolsky, 2003). Furthermore, TFFs have been proposed as inflammatory mediators and connected with a possible role in immune regulation (Baus-Loncar et al., 2005). It was reported that serum concentrations of human TFF1, TFF2 and TFF3 were significantly increased (200 pM) in inflammatory bowel disease patients (Gronbaek et al., 2006). However, key questions remain to be resolved to achieve a full understanding of the first-hand actions of TFFs and the molecular mechanisms involved (Taupin and Podolsky, 2003).

βγ-CAT is the first example of a naturally existing non-lens βγ-crystallin and trefoil factor complex characterized from the Chinese red belly frog (*Bombina maxima*) skin secretions (Liu et al., 2008). Its α- and β-subunits, with a non-covalently linked form of $\alpha\beta_2$, show significant sequence homology to human AIM1 (Ray et al., 1997) (Fig. 1) and TFFs (Tomasetto et al., 1990; Hauser et al., 1993; Zhang et al., 2005), respectively (Liu et al., 2008). In our previous study (Qian et al., 2008), it was shown that βγ-CAT possessed potent lethal toxicity on mammals resulted from hypotension and cardiac inhibition. However, the exact mechanism of the cardiotoxicity of βγ-CAT is still unknown. In this paper, we report that βγ-CAT elicited an acute negative inotropic effect on isolated rabbit hearts, which mimicked acute heart failure. In addition, this cardiotoxic effect was endothelium-dependent. The rapid and significant release of tumor necrosis factor-α (TNF-α) was detected both *in situ* of the organ and in primary cultured rabbit endocardial endothelial cells (REECs), which might be partially responsible for the myocardial depression caused by βγ-CAT.

2. Materials and methods

2.1. Animals and ethics

Adult male New Zealand rabbits (2–3 kg) were provided by the Animal Center of the Kunming Medical College. Animal care and handling were conducted in accordance with policies on the care and use of animals promulgated by the Ethics Committee of Kunming Institute of Zoology, The Chinese Academy of Sciences.

2.2. Purification of βγ-CAT

The purification of βγ-CAT was followed as described previously (Liu et al., 2008). Briefly, the lyophilized frog *B. maxima* skin secretions (from a stock in Kunming Institute of Zoology) were dissolved in 10 ml of 50 mM Tris–HCl buffer, pH 7.3, containing 5 mM EDTA, dialyzed against the same buffer at 4 °C overnight and centrifuged. The supernatant was loaded on a set of DEAE Sephadex A-50 (Pharmacia), Sephadex G-100 (Pharmacia, superfine) and AKTA Mono-Q HR5/5 anion ion exchange columns, and eluted as described previously. Protein concentration was

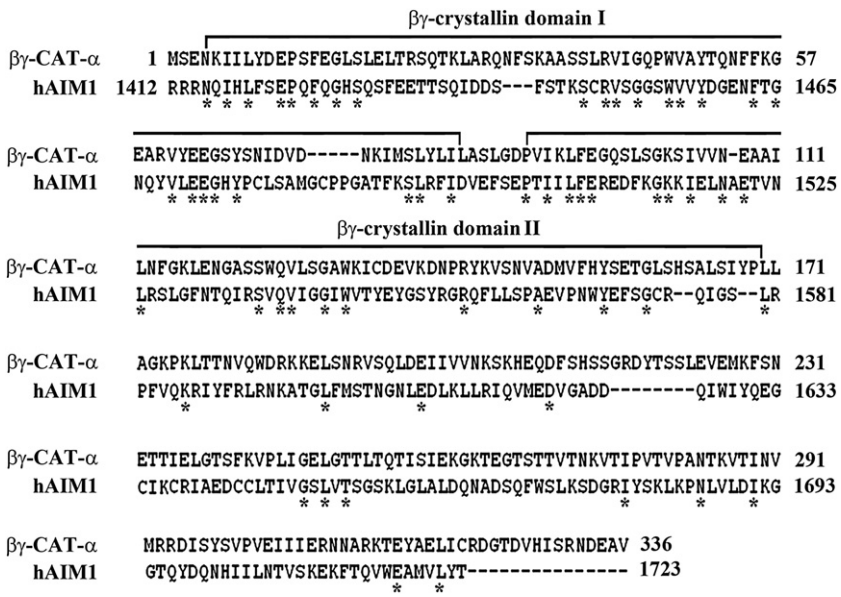

Fig. 1. Sequence comparison of βγ-CAT α-subunit (Liu et al., 2008) with βγ-crystallin domains 5-6 plus C-terminal part of human AIM1 (Ray et al., 1997). The characteristic βγ-crystallin domains are marked. The identical residues are shown by asterisks. Gaps have been introduced to optimize the sequence homology.