General and Comparative Endocrinology 240 (2017) 1–9

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Research paper

The Chromogranin A-derived sympathomimetic serpinin depresses myocardial performance in teleost and amphibian hearts

S. Imbrogno [a,*,1], R. Mazza [a,1], C. Pugliese [a], M. Filice [a], T. Angelone [a], Y.P. Loh [b], B. Tota [a], M.C. Cerra [a]

[a] Dept. of Biology, Ecology and Earth Sciences (B.E.S.T.), University of Calabria, Arcavacata di Rende (CS), Italy
[b] Section on Cellular Neurobiology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

ARTICLE INFO

Article history:
Received 13 June 2016
Revised 5 September 2016
Accepted 11 September 2016
Available online 12 September 2016

Keywords:
β3-Adrenoceptors
Cardiac performance
cGMP
ET_B receptors
Negative inotropism
Sympatho-adrenergic signaling

ABSTRACT

Chromogranin A (CgA) is an acidic protein co-stored with catecholamines, hormones and neuropeptides in the secretory granules of endocrine, neuronal and other cell types (including cardiomyocytes). Proteolytic cleavage in the C terminus of CgA generates a 2.9 kDa peptide named serpinin (Serp; Ala26Leu) that can be modified at its N terminus to form a pyroglutamate residue (pGlu-Serp). In the rat heart, both peptides increase contractility and relaxation through a β-adrenergic-like action mechanism. Accordingly, Serp and pGlu-Serp were proposed as novel myocardial sympatho-adrenergic modulators in mammals.

On a comparative basis, here we report the actions of Serp and pGlu-Serp on myocardial contractility in three poikilotherm vertebrate species: the eel (*Anguilla anguilla*), the goldfish (*Carassius auratus*) and the frog (*Rana esculenta*).

Using isolated working heart preparations, we show that pGlu-Serp reduces stroke volume in all species tested, while Serp reduces contractility in the frog heart, but is uneffective in eel and goldfish hearts. In the goldfish and frog hearts, pGlu-Serp activates the Nitric Oxide/cGMP pathway involving Endothelin-1 B receptors (frog) and β_3 adrenergic receptors (goldfish). pGlu-Serp-treated hearts from goldfish and frog show increased cGMP content. Moreover, the exposure of the frog heart to pGlu-Serp is accompanied by an increased expression of activated eNOS and Akt.

In conclusion, this first report showing that pGlu-Serp inhibits mechanical cardiac performance in teleost and amphibians supports an evolutionary role of the CgA system, and particularly its serpinin component, in the sympatho-adrenergic control of the vertebrate heart.

1. Introduction

Chromogranin A (CgA) is a major acidic protein of the secretory granules of the diffuse sympatho-chromaffin tissue (Winkler and Fischer-Colbrie, 1992) and is also present in endocrine, nervous and other cell types, including cardiomyocytes (Helle et al., 2007). It is co-stored in and co-released from the granules together with catecholamines (CAs), several hormones and neuropeptides (Winkler and Fischer-Colbrie, 1992). Identified almost five decades ago, CgA was extensively studied as to its expression, structure and function. The long evolutionary history of CgA is evidenced by its occurrence in many invertebrates such as coelenterates (Barkatullah et al., 1997), the nematode parasite *Ascaris suum* (Smart et al., 1992) and the protozoan *Paramecium tetraurelia* (Peterson et al., 1987). In vertebrates, CgA is ubiquitous and highly conserved. It is present in teleost fish (Deftos et al., 1987), amphibians (Reinecke et al., 1991), reptiles (Trandaburu et al., 1999), birds (Reinecke et al., 1991), humans, pigs and rats (Tota et al., 2007; Helle et al., 2007). CgA-positive cells have been detected in a number of mammalian species during early developmental stages in the adrenal medulla and the gastro-entero-pancreatic system together with various endocrine cells (Kent and Coupland, 1989; Mahata et al., 1993; Wang et al., 1994; Kameda et al., 1998). CgA-positive cells are also present in the brain and the dorsal root ganglia of the zebrafish (*Danio rerio*) (Xie et al., 2008), suggesting a

Abbreviations: β-ARs, β-adrenergic receptors; BSA, bovine serum albumin; CAs, catecholamines; CgA, Chromogranin A; CO, cardiac output; CST, Catestatin; ET-1, Endothelin-1; ET_AR, ET-1 type A receptors; ET_BR, ET-1 type B receptors; GC, guanylyl cyclase; HR, heart rate; NO, Nitric Oxide; NOS, Nitric Oxide Synthase; pAkt, phospho-Akt; peNOS, phospho-eNOS; pGlu-Serp, pyroglutamate serpinine; PI3K, PI3kinase; PKA, cAMP-dependent kinase; PKG, protein kinase G; PTx, pertussis toxin; Serp, serpinin; SV, stroke volume; SW, stroke work; VSs, Vasostatins.

* Corresponding author at: Lab of Organ Physiology, Dept. of Biology, Ecology and Earth Sciences, University of Calabria, 87036 Arcavacata di Rende (CS), Italy.
E-mail address: sandra.imbrogno@unical.it (S. Imbrogno).

[1] These authors equally contributed to the work.

http://dx.doi.org/10.1016/j.ygcen.2016.09.004
0016-6480/

role during the early embryonic stages. Of cardiovascular relevance, studies performed during the last twenty-five years showed the presence of CgA also in the heart of a number of vertebrates. In the rat, it was identified in atrial myoendocrine granules (Steiner et al., 1990) and in the cardiac conduction system (Weiergraber et al., 2000). In humans, CgA was detected in the ventricular myocardium (Pieroni et al., 2007). Both in the human and rodent heart, it was found to be co-stored and co-secreted with CAs and natriuretic peptides (Steiner et al., 1990; Pieroni et al., 2007; Biswas et al., 2010). In the heart of non-mammalian vertebrates, CgA expression was detected in the secretory granules of frog atrial myocytes (Krylova, 2007).

CgA is a multifunctional protein which plays important biological roles. Within the (neuro)endocrine cells, in addition to the regulation of granule biogenesis (Courel et al., 2006), CgA functions as a prohormone for several biologically active peptides that control multiple homeostatic processes. These include the CgA-derived fragments Vasostatins (VSs) and Catestatin (CST) whose role as anti-adrenosympathetic stabilizers in cardiovascular homeostasis and anti-ischemic cardioprotection is under intensive investigations. VSs, the fragments corresponding to amino acids 1–76 (VS-1) and 1–113 (VS-2), and CST, a COOH-terminal fragment, in addition to their vascular effects, are now recognized as important cardiotropic modulators. In fact, in both mammalian and non mammalian vertebrates they exert anti-adrenergic cardiosuppressive actions for which they have been proposed as cardio-protective agents able to counteract the effects of excessive systemic and/or intra-cardiac excitatory stimuli [e.g. CAs and Endothelin-1 (ET-1)] (Tota et al., 2003; Corti et al., 2004; Imbrogno et al., 2004, 2010; Cerra et al., 2006; Angelone et al., 2008; Mazza et al., 2008, 2015a).

More recently, it has been shown that CgA cleavage at paired basic residues in its highly conserved C terminus, gives rise to a 2.9 kDa peptide named Serpinin (Serp; Ala26Leu) that can be modified at its N terminus to form a pyroglutamate residue (pGlu-Serp) (Koshimizu et al., 2011a,b). In neuroendocrine cells, Serp upregulates granule biogenesis *via* a cAMP-protein kinase A-Sp1 pathway (Koshimizu et al., 2011a), while pGlu-Serp inhibits cell death (Koshimizu et al., 2011b). Both Serp and pGlu-Serp have been proposed as novel CgA-derived cardiac modulators able to play a role in the myocardial response to sympathochromaffin stimulation. In fact, Tota and co-workers (2012) detected both fragments in the rat heart and used an *ex vivo* rat heart, Langendorff isolated and perfused preparation, to demonstrate that Serp and pGlu-Serp exert dose-dependent positive inotropic and lusitropic effects. Unlike the other CgA-derived cardioactive fragments (i.e. VS-1 and CST), which are well known Nitric Oxide (NO)-dependent negative anti-β adrenergic inotropes, both peptides activate a β1-adrenergic receptor (β1-AR)/adenylate cyclase (AC)/cAMP/cAMP-dependent kinase (PKA) pathway, acting as β-adrenergic-like agonists (Tota et al., 2012).

Very recently, employing the same rat heart preparation, Pasqua and co-workers (2015) have investigated the anti-ischemic cardioprotective potential of pGlu-Serp used in either pre-conditioning and post-conditioning experiments, showing in both conditions its striking implication in limiting ischemia-induced infarct size and myocardial failure.

In an evolutionary perspective, and with the aim to comparatively analyse the role of Serpinin peptides, we explored whether Serp and pGlu-Serp also affect myocardial contractility in three poikilotherm vertebrate species, namely the eel *A. anguilla*, the goldfish *C. auratus* and the frog *R. esculenta*.

We demonstrated that pGlu-Serp induces a reduction of contractility in all species tested. The pGlu-Serp-elicited effects appear mediated by a $G_{i/0}$/Akt/Nitric Oxide Synthase (NOS)/NO/cGMP/protein kinase G (PKG) signal transduction pathway and involve β3-ARs in goldfish and ET-1 B receptors (ET_BR) in frog. Taken together, these data suggest an early role of the CgA C-terminal Serpinin fragments in vertebrates, also highlighting the importance of species-specific action mechanisms.

2. Materials and methods

2.1. Animals

Specimens of eel (*A. anguilla* L.), goldfish (*C. auratus*) and frog (*R. esculenta*) of both sexes (body weight 97 ± 7 g, 55 ± 3 g and 21 ± 2 g, respectively) were used. Eels and frogs were provided by local hatcheries, while goldfish were provided by a fish farm (COF SAS, Bologna, Italy). All animals were kept at room temperature (18–20 °C) for 7–10 days. In accordance with the accepted standards of animal care, the experiments were organized to minimize stress and number of animals used. Animal care and procedures were in accordance with the U.S. National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996), with the Italian law (DL 26, March 4, 2014), and with Directive 2010/63/EU.

The experiments evaluating the effect of Serpinin peptides on the basal cardiac performance of the eel (*A. anguilla*) were performed in 2013. However, following the new Italian law about the care and use of Laboratory Animals, effective from March 2014, in which the European eel is considered an endangered species, this animal was excluded from further experiments designed to investigate the mechanism of action of the peptides.

2.2. Isolated and perfused in vitro working heart preparations

Eel and goldfish were anesthetized in benzocaine (0.2 g/L) for 15 min. After sacrifice, the heart was dissected out, removed by the pericardium, cannulated and connected with a perfusion apparatus (Imbrogno et al., 2001; Garofalo et al., 2012). For the eel heart, the perfusate composition (in mM) was: 115.17 NaCl, 2.03 KCl, 0.37 KH_2PO_4, 2.92 $MgSO_4$, 0.5 $(NH_4)_2SO_4$, 1.27 $CaCl_2$, 5.55 glucose, and 1.90 Na_2HPO_4 (Imbrogno et al., 2001). The goldfish heart was perfused with a Ringer's solution containing (in mM): NaCl 124.9, KCl 2.49, $MgSO_4$ 0.94, NaH_2PO_4 1, Glucose 5, $NaHCO_3$ 15, and $CaCl_2$ 1.2 (Garofalo et al., 2012). In both cases, saline was equilibrated with a mixture of 99.5% O_2 and 0.5% CO_2 and the pH was adjusted to 7.7–7.9 by adding $NaHCO_3$. Frogs were pithed and ventrally opened; the pericardium was removed and the heart, cannulated *in situ*, was connected to a perfusion apparatus (Gattuso et al., 1999). Frog Ringer solution contained (in mM): 115 NaCl, 2.5 KCl, 1.0 $CaCl_2$, 2.15 Na_2HPO_4, 0.85 NaH_2PO_4, and 5.6 glucose. Saline was equilibrated with air. pH was adjusted to 7.3 by adding Na_2HPO_4.

Experiments were carried out at room temperature (18–20 °C). Hemodynamic parameters were measured as previously reported (Gattuso et al., 1999; Imbrogno et al., 2001; Garofalo et al., 2012). Cardiac output (CO) was collected over 1 min and weighed. Values were corrected for fluid density and expressed as volume measurements. Heart rate (HR) was obtained from the periodicity of pressure traces. Stroke volume (SV = CO/HR) was used as a measure of ventricular performance. Ventricular stroke work [SW; mJ/g; (afterload-preload) × SV/ventricle mass] served as index of systolic functionality.

2.3. Experimental protocols

2.3.1. Basal conditions

Isolated and perfused hearts were allowed to maintain a spontaneous rhythm for up to 15–20 min. In all experiments, the control conditions were: mean output pressure 3.00 kPa with CO set