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Co-purification of arrestin like proteins with alpha-enolase from bovine myocardial tissues and the possible role in heart diseases as an autoantigen

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ABSTRACT

Aim: Previously, we reported that visual arrestin co-purified with glycolytic enzymes. The aim of this study was to analyze the co-purification of arrestin like proteins (ALP) in bovine cardiac tissues with enolases.

Methods: The soluble extract of bovine myocardial tissues from different regions such as left and right atriums and ventricles of the bovine heart (n = 3) was analyzed by ACA-34 gel filtration, immuno-affinity column, SDS-PAGE, ELISA, western blot and a sandwich immune assay for quantification of ALP and sequence analysis.

Results: We observed that; 1) The cardiac muscle contained a 50 kDa ALP at a concentration of 751 pg/mg of soluble protein extract, 2) ALP purified, by immunoaffinity, contained alpha-enolase of 48 kDa confirmed by protein sequence analysis; 3) Cardiomyocyte cells exposed to anti arrestin and anti enolase monoclonal antibodies showed decreased proliferation *in vitro*, 4) High level of autoantibodies were detected by ELISA (3.57% for arrestin and 9.12% for α-enolase) in serum of patients with infarcted heart disease.

Conclusion: We suggest a possible interaction between ALP and alpha-enolases yielding a complex that may be involved in the induction of cardiac autoimmune diseases.

1. Introduction

Arrestins play a central role in desensitization of G-protein-coupled receptors (GPCRs) [1,2]. G protein coupled receptors (GPCRs) use two major signaling mechanisms, one mediated through the classical activation of G-proteins and the other through the activation of β arrestins [3]. β arrestin, in addition to receptor desensitization, is involved in receptor endocytosis, activation of extracellular signal regulated kinases (ERK) and other mitogen activated protein (MAP) kinases [4]. β arrestin which is an isoform of arrestin, form complexes with several signaling proteins, including Src family tyrosine kinases and components of the ERK1/2 and JNK3 MAP kinase cascades [1]. We and others have shown that immunization of mammals by arrestin (S-antigen) as an auto antigen induces experimental uveitis [5]. Cardiac muscle cells harbor an important family of surface protein receptors GPCRs which enables cells to sense and respond to outside signals [6].

Another protein, enolase, is a glycolytic cytoplasmic enzyme, known to be a multifunctional and cell membrane α-enolase has been identified as plasminogen receptor (PR) [7]. Wound healing after infarct failed in plasminogen-deficient mice, indicating that the plasminogen-R system is required for the repair process of the heart post infarction [8]. In *in vitro* studies, α-enolase was found necessary for regeneration of normal and dystrophic skeletal muscles [9]. In rat, α-enolase was found to be strongly induced in response to ischemic hypoxia [10], it has therefore been proposed as a marker for early diagnosis of acute myocardial infarction [11].

A few years back, we had reported that bovine visual arrestin co-purified with selected glycolytic enzymes such as α and γ enolase, aspartate aminotransferase and glucose-6-phosphate isomerase [12]. We had also demonstrated the presence of arrestin likes proteins (ALP) in cardiac tissues [13]. In the present work, we show using (anti visual arrestin specific monoclonal antibodies immunoaffinity column), that ALP from bovine cardiac muscle extracts

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co-purified with α-enolase. Because of the pathogenic potential of visual arrestin in uveitis and arrestin-enolase in neuronal tissues as auto antigens [14], the serum from patients with heart diseases were tested for the presence of auoantibodies against both these proteins. Since these proteins may be involved in the induction of cardiac autoimmune diseases.

2. Materials and method

2.1. Antigen and antibodies

Visual S-antigen (arrestin) was purified from bovine retina as described [15] Polyclonal anti arrestin was prepared by injecting rabbits with 200 μg of protein emulsified in complete Freund's adjuvant [5]. Several mouse and rat monoclonal antibodies (mAbs) against retinal arrestin were produced [16] and characterized by epitope mapping employing combinations of two methods, namely, overlapping of synthetic peptides and petscan analysis as described previously [17]. Antibodies S8D8 (mouse IgG2a aa 40–50), S9E2 (mouse IgG2a, aa 361–368) and rat mAb M1E5 (rat IgG1, aa 279–306) were produced as previously reported [16,18]. On western blot of crude extracts from mammalian retinas, these mAbs reacted with a single band of 48 kDa. Monoclonal anti enolase (PR11G-1 IgG1) was retired in our laboratory by immunization of mouse using MCF-7 breast adenocarcinoma cell line, as previously described [19]. E1A3 another anti enolase produced when mouse anti-retinal arrestin were prepared. All anti enolase antibodies reacted with purified enolase and recognized a band at 48 kDa in western blots.

2.2. Bovine myocardial tissue extracts

Crude soluble extracts were prepared according to the following procedure. Myocardial tissues from fresh bovine hearts were dissected free from endocardial tissues. Pooled myocardial tissues or left/right atriums and left/right ventricles were prepared separately. Seventy grams of tissues were homogenized at +4 °C in presence of antiprotease phenylethylsoufonyl fluoride with an Ultra turrax tissue grinder in 140 mL of ice-cold hypotonic 10 mM phosphate buffer, pH 7.4. It was then centrifuged at 27×10^3 g for 30 min at 4 °C. We prepared 12 different myocardium soluble extracts (see Table 1) from which arrestin like proteins were quantified.

2.3. Purification of heart arrestin like proteins (ALP)

An equal volume of saturated ammonium sulfate was added to the crude extracts described above. The resulting precipitate was dissolved In 10 mM phosphate buffer solution (PBS), pH 7.4, dialyzed against PBS, centrifuged and fractionated by gel filtration on an ultrogel ACA-34 (Sigma–Aldrich, France) column. The fraction containing immunoreactive protein detected by ELISA were pooled and passed through monoclonal anti retinal arrestin antibody (S8D8) immunoaffinity Column. The bound material was eluted with 5M Tris, 5M urea buffer, pH 9.5 and dialyzed against PBS, pH 7.4. They were than tested in an ELISA using the rabbit anti arrestin antibody and monoclonal anti arrestin antibodies. The positive fractions were pooled and concentrated under vacuum. Protein characterization was performed by gel electrophoresis. The same method was used to purify the immunoreactive proteins of the cardiac soluble extract using columns of immobilized rabbit anti arrestin antibody. In this experiment the bound material was desorbed by 200 mM glycine-HCL buffer, pH 2.5, at 4 °C and the eluted fractions were immediately neutralized using 2M Tris the purified fraction were analyzed by electrophoresed for 4 h in a 15% acrylamide.

2.4. Quantification of arrestin like protein

Monoclonal anti arrestin (S8D8) was used as solid phase (capture antibody) and rabbit polyclonal antibodies as conjugates (enzymatic tracer). Conjugates were prepared by coupling the tetrameric form of the acetylcholinesterase enzyme (ACHE) (EC 3. 1. 1. 7 from electrophorus electricus, G4 form) with antibody. 96 well microplates were coated with S8D8 (10 μg/ml) overnight at 4 °C in sodium bicarbonat buffer–Phosphat buffer (PH 9.5). After 3 washes with PBS the plates were reacted either with bovine standard retinal arrestin in 0.01M sodium phosphate buffer, pH 7.0, containing 0.10% BSA, or with different myocardium extracts diluted in the same buffer together with the enzymatic conjugate after overnight immunoreactions at 4 °C. Plates were washed and immobilized AChE activity was measured as described [20]. Protein determination was performed by Bradford technique.

2.5. Western blot

Proteins of immunopositive fractions were denatured in 220 mM Tris–HCl buffer, pH 6.0, with 2% SDS, 15% glycerol and electrophoresed for 4 h in a 10–20% acrylamide gradient gel and then electrotransferred to nitrocellulose membranes (Millipore, Bedford, MA) for 1 h in 0.15% Tris–HCl, 0.72% glycine, 20M (v/v) methanol. The membrane was incubated with (5 μg/ml) polyclonal or monoclonal antibodies followed by their detection with biotinylated goat anti-rabbit or sheep anti-mouse. A series of standards (Bio-rad, France) were used as molecular mass.

2.6. Sequence analysis

was performed as previously described [12]. Briefly, Following SDS-PAG and electroblotting, of purified arrestin like protein by S8S8 monoclonal anti arrestin antibody, the blotted spot, 48 and 50 kDa, were cut into small pieces and triyptic digestion was performed according Aebersold [21]. The resulting peptides were separated by revers phase HPLC followed by acetonitrile. Sequence determination was performed by Edman degradation [22] with an Applid Biosystem 473 sequencer [23] and the sequences were compared with those in the NBRF bank, using the FASTA program.

2.7. ELISA

ELISA was performed for detection of arrestin and enolase immunoreactivity in all fraction of chromatography as described in [12]. In order to determine the role of the serum anti arrestin/enolase as a possible indicator in heart autoimmunity, we conducted a retrospective study on 157 patients with myocardial infarcts (from Pitié-Salpêtrière University Hospital, Paris, France, Pr. J. P Collet). The detection of auto antibodies by ELISA, against target proteins used 0.2 mL of plasma for each patient.

2.8. Cell proliferation

3000 myocardial cells (H9C2; rat cell line) in DMEM complemented by 10% calf fetal serum was plated in E-plate 96 (Roche, San Diego, USA) and placed in a wet xCELLigence apparatus (Roche, France) at 37 °C, 5% CO_2 for real time cell proliferation assay. After 48 h, the cells in E-plate were treated with a fresh complete DMEM medium containing 5-μg of different monoclonal antibodies (anti arrestin and anti enolase). The xCELLigence System measures electrical impedance across interdigitated micro-electrodes

integrated on the bottom of tissue culture E-Plates and monitors cellular events in real time without the incorporation of labels.

3. Results

3.1. Bovine myocardial tissues extracts contain arrestin like proteins

Fig. 1A shows the gel filtration profile of soluble proteins from myocardium. A series of fractions reacted positively giving a major pick in ELISA against monoclonal antibody S8D8 (Peak A, fractions 58–76). Crude tissue extract showed a weak immunoreactivity with antibodies to retinal arrestin (results not shown). The pooled peak "A" from gel filtration was passed through an immunoaffinity column of S8D8 Mab (Fig. 1B). Fig. 1C shows the SDS-Page analysis of pooled fractions from Peak A of crude myocardial extracts (C-1), hydroxyl apatite purified bovine visual arrestin of 48 kDa (C-2) and Immunopurified (fractions 41–55) using rabbit anti arrestin revels an arrestin band of superior molecular weight, 50 kDa as compared to visual arrestin (C-3). Fig. 1C (C-4) shows western blot analysis of pooled fractions 41-55 from immunoaffinity column with S8D8 Mab compared with the control (F1E1 mab) presented in Fig. 1C (C-5). The four monoclonal anti arrestin antibodies (S2D2, S8D8, M1E5, S9E2) reacted positively by ELISA with pooled fractions 41-55 as compared to the control antibody (F1E1).

3.2. Quantification of ALP in soluble extracts of bovine myocardium

In the 4 regions of bovine hearts (n = 3) such as left and right atriums and ventricles, arrestin like proteins were detected. As presented in Table 1, the total protein concentrations of all extracts were comparable irrespective of which region of the heart and that to all the three hearts. The concentration of immunoreactive proteins in the crude myocardium soluble extracts was approximately 0.75 μg per gram of protein. The soluble extract contained 13.6 ng/ml of arrestin like proteins per 18.1 mg/ml of soluble extract (ratio 1/1330). Conversely, there was a large variation in the amount of arrestin like immunoreactive proteins between the four compartments of the same heart, ranging from 0.11 to 2.43 μg/g proteins for a given compartment in the three hearts.

Table 1
Quantification of arrestin like proteins in the 4 regions of the heart. In the 4 regions of the heart such as left and right atriums and ventricles of the 3 bovine hearts, soluble extract contained 13.6 ng/ml (46.83–2.02 ng/ml) of arrestin like proteins per 18.1 mg/ml (21.6–12 mg/ml) of soluble extract (ratio 1/1330). The data suggest that the cardiac muscle contained arrestin like protein at a concentration of 751 pg/mg of soluble extract protein.

Cardiac muscle extract	Total protein mg/ml	Arrestin like proteins ng/ml
RA1	15.61 ± 2.93	3.58 ± 0.44
RA2	14.96 ± 3.17	8.05 ± 0.57
RA3	18.53 ± 4.32	18.79 ± 3.15
LA1	19.29 ± 4.21	46.83 ± 8.56
LA2	21.60 ± 4.57	16.83 ± 2.84
LA3	18.31 3.16	4.07 ± 0.53
RV1	18.95 ± 3.77	5.91 ± 0.77
RV2	18.87 ± 2.94	11.40 ± 1.21
RV3	15.57 ± 3.62	26.86 ± 3.24
LV1	18.42 ± 3.14	2.02 ± 1.22
LV2	18.49 ± 3.71	2.88 ± 041
LV3	18.50 ± 3.26	17.49 ± 3.62
RA pool	17.72 ± 3.31	9.94 ± 1.22
LA pool	18.70 ± 3.20	19.93 ± 4493
Rv pool	19.64 ± 4.17	13.82 ± 3.08
Lv pool	16.63 ± 3.54	7.87 ± 0.86

3.3. Co-purification of ALP with α-enolase

Western blot analysis of the ALP, immune-purified from the soluble extract of myocardial tissues fractions of ACA-34 gel filtration, contained ALP immunoreactivity. The results confirm the presence of a 50 kDa protein that reacts with anti arrestin (S8D8 Mab, Fig. 2A,1) anti α-enolase (PR-11G1 Mab, Fig. 2A, 2), polyclonal rat anti-bovine heart anti-ALP (Fig. 2A, 3). It reveals a double band corresponding to 50 and 48 kDa representing arrestin and its co-purified protein, α-enolase. Similar results were obtained with the fractions retained by immunoaffinity using other anti arrestin

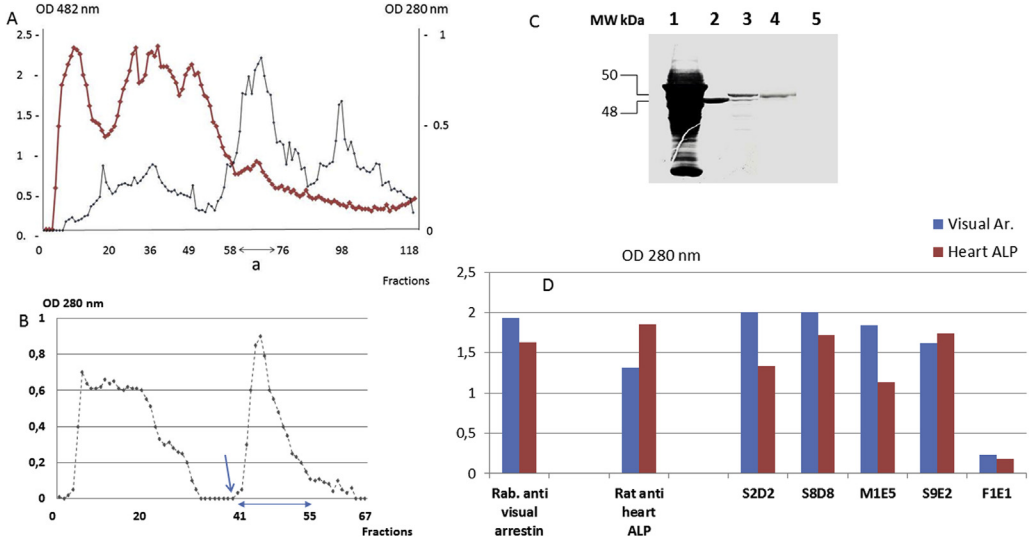

Fig. 1. The soluble extract of bovine's myocardial tissues analysis. A: The soluble extract of bovine's myocardial tissues analyzed by ACA-34 gel filtration and arrestin like immunoreactivity reveled using anti arrestin antibody, A major peak (peak a, fractions of 61–76) contain arrestin like protein. B: Peak a analyzed by S8D8 immunoaffinity column, the fraction of 41–55 contained ALP. C: SDS-PAG analysis of all fraction Myocardial extracts (a) HA purified arrestin (b), Immunopurified heart ALP by immunoaffinity by a rabbit anti arrestin (c) and using S8D8 monoclonal antibody (d). A 50 kDa arrestin like proteins purified from myocardium soluble extract by anti arrestin antibodies (SDS-PAGE 10%). D: EIISA analysis of immunopurified heart ALP by several monoclonal antibodies to retinal arrestin.

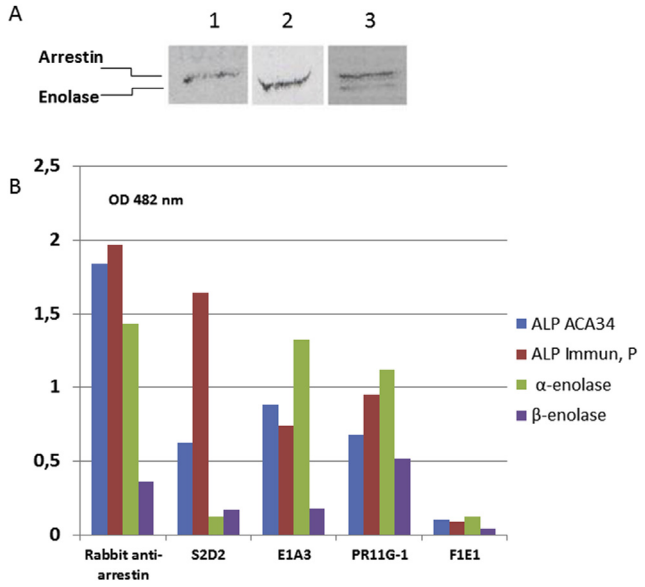

Fig. 2. Cross reaction of anti arrestin and anti α-enolase monoclonal antibodies with cardiac arrestin purified by S8D8 immunoaffinity. A: by anti arrestin (a), anti enolase (b) and rat anti heart ALP (C). B: Elisa using rat anti arresti, S2D2 (anti arrestin), E1A3, PR11G1 (anti enolase) and F1E1a monoclonal antibody to fibrinogen (control).

monoclonal antibodies or rabbit polyclonal antibodies to retinal arrestin (results not shown). These results were confirmed when ALP from immunoaffinity column was tested in ELISA with several antibodies against visual arrestin (S2D2), and monoclonal anti α-enolase antibodies (E1A3 and PR11G1) (Fig. 2B). F1E1 an anti-human fibrinogen, was used as control.

3.4. Sequences analysis

Structural analysis of two tryptic digest proteins (Table 2) reveled four peptides for 48 kDa, and 3 for 50 kDa proteins with the following sequences: The peptides (KLMIEMDGT), (LAMQEFM), (KNVIKEK) and (VVGLCTGQIK) were identified for 48 kDa band and (KRDFVDHI), (KLKHEDTNLA) and (GDLSSD) for 50 kDa band. These

Table 2
Sequences analysis of trypsin depredate products of 50 kDa and 48 kDa bands. Three peptides (F1 to F3) in digested 50 KDa protein reveled the strict homology b arrestin family(A) and four peptides (F1 o F4) in digested 50 KDa protein reveled the strict homology with α-enolase.

A	
Trypsin depredate products of 50 kDa	Polypeptide sequences
F-1	KRDFVDHI
β arrestin	24-KRDFVDHI-33
F-2	KLKHEDTNLA
β arrestin	291-KLKHEDTNLA-301
F-3	GDLSSD
β arrestin	336-GDLSSD-343
B	
Trypsin depredate products of 48 kDa	Polypeptide sequences
F-1	KLMIEMDGT
α-enolase	91-KLMIEMDGT-101
F-2	LAMQEFM
α-enolase	163-LAMQEFM-171
F-3	KNVIKEK
α-enolase	193-KNVIKEK-201
F-3	VVGLCTGQIK
α-enolase	285-VVGLCTGQIK -296

results confirm that using anti-pan arrestin monoclonal antibodies for purification of arrestin, enolase will be co-purified with ALP.

3.5. The effect of antibodies against arrestin and enolase on cardiomyocyte cell proliferation

The proliferations of cells during 48 h after elimination of antibodies from culture media were measured using x*CELLigence* apparatus and the results presented as cell (proliferation index) in Fig. 3. Fig. 3 shows the myocardyocyte proliferation in culture (control). This proliferation was affected when anti arrestin S8D8 (epitope localized in N-terminal) or S9E2 (epitope localized in C-terminal) or anti enolase PR11G-1 was added to the culture medium. Using x*CELLigence* system, reveled information during the progression of the experiments will be recorded and known in real time.

3.6. Autoantibody detection against of the arrestins and the enolases in patients with heart disease

The serums of patients with the coronary heart disease were tested for detection of auto antibodies against retinal arrestin and α-enolase. High level of autoantibodies was found in some of the patients tested against of bovine retinal arrestin and α-enolase (Table 3). The immunoractivity of serum with the proteins in ELISA, divided the patients in four categories: category A- 9.12% serum contained antibodies against α-enolase, category B- 3.57% serum contained antibodies against arrestin, category-C 1.9% serum contained antibodies against arrestin and α-enolase and category-D 85.4% absence of immunoreactivity. These results point out that auto antigen are detectable by ELISA in human cardiac infarcts.

4. Discussion

Arrestin like proteins from the bovine myocardium soluble extracts were purified by immune affinity columns using a monoclonal antibody that recognized an epitope presented in all members of arrestin family (40-50 aa epitope in bovine visual arrestin). Eluted proteins comprised 2 proteins components with molecular weights of 48 and 50 kDa. As verified by western blot, ELISA, and protein sequencing, the 48 kDa turn out to be the alpha-enolase and the 50 kDa as arrestin. This result indicated that, arrestin family was completed with enolase in the heart muscles.

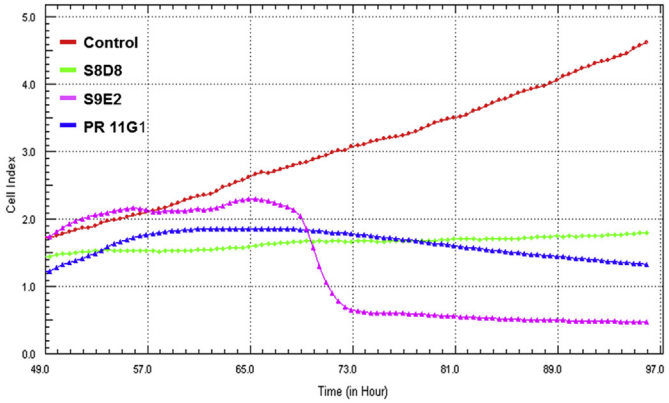

Fig. 3. The effect of antibodies on the rat cardiomyocyte cell line proliferation. 5 ug/ml of monoclonal antibodies; anti-arrestin S8D8 (anti N-terminal), anti-arrestin S9E2 (anti C-terminal), anti-enolase PR11G1 were added in culture medium for 48 h. The real time proliferations of the cells during 48 h after elimination of antibodies from culture media were measured using x*CELLigence* apparatus and results presented as cell (proliferation) index.

Table 3
The immunoractivity of serum with the proteins, bovine visual arrestin and rabbit α-enolase in ELISA were different according to patients. group A, serum contained antibodies against arrestin, group B, serum contained antibodies against α-enolase, group C, serum contained antibodies against arrestin and α-enolase and group D, absence of immunoreactivity.

Patients	Arrestin (OD 482 nm)	Enolase (OD 482 nm)
A (3.57%)		
301	1191	0.150
241	1628	0.137
B (9.12%)		
281	0.042	0.885
213	0.255	0.996
C (1.9%)		
320	0.799	0.568
47	0.615	0.650
D (85.4%)		
323	0.044	0.033
335	0.051	0.097

Arrestin and α-enolase are cytosolic proteins, whereas the membrane associated enolase, described earlier by our group [24] and by other authors, as plasminogen-receptors [24,25] The occurrence of arrestin in the membrane bound form on the external cell surface is not so far documented.

The bovine heart (consisting of the four regions left and right atriums and left and right ventricles) contained 13.6 ng/ml of arrestin like proteins per 18.1 mg/ml of soluble extract (ratio 1/1330). The data indicates that bovine cardiac muscles contain arrestin like protein at a concentration of 751 pg/mg of total soluble protein extract. Since we are looking at soluble heart protein extracts, this study confines itself to only free cytosolic arrestin. The content of arrestin varies in the four different regions of the heart. Surprisingly, left atrium (LA) and right ventricle (RV) cardiac muscles contain more soluble arrestin (free) compared to right atrium (RA) and left ventricle (LV) (Table 1). The membrane bound arrestin present in RA and LV are not available for evaluation.

From the data generated In this study we are tempted to speculate that in pathologic condition, arrestin (and enolase)- no resensitizated GPCRs complex can be externalized as an auto-antigen on the cardiomyocyte membrane which could result in the induction of autoantibodies (Fig. 4C).

We had demonstrated earlier the co-purification of visual arrestin with glycolytic enzyme such as α and γ enolase, aspartate aminotransferase and glucose-6-phosphate isomerase [12]. Enolase, also known as phosphopyruvate hydratase, is one of the most abundantly expressed cytosolic proteins in many organisms. It is a metallo-enzyme that requires the magnesium ions (Mg^{2+}) for its catalytic activity [26]. Three isoforms of enolase; α-enolase present in all tissues, β-enolase present in muscles and γ-enolase in neurons were described in all vertebrate. Tissue specific isozymes of enolase (β and γ) readily form mixed hetro dimers with α-enolase [26]. In this study, we observed the interaction of arrestin like proteins only with α-enolase. Whether, or not, β and γ enolases also interact with heart arrestins remains yet to be demonstrated.

Serum from patients with the heart disease was tested for the presence of arrestin-enolase auoantibodies. We tested by ELISA the serum of 157 infarcted heart diseases, and found a high level of autoantibodies (3.57% for arrestin and 9.12% for α-enolase) compared with other serum. The relation between the autoantibodies detected and heart disease could not be clarified. Though all the sera came from infarct cases the distinctive features of individual samples could not be taken into account. It remains to be seen whether these antibodies are disease specific. The appearance of autoantibodies would have deleterious effect on cardiac cells.

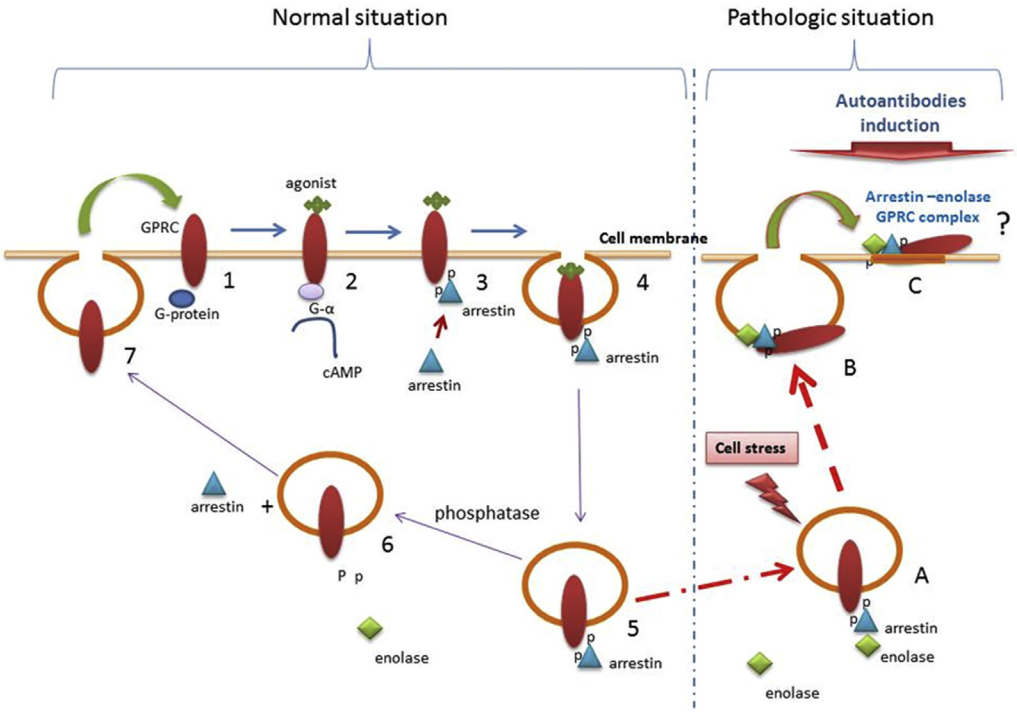

Fig. 4. a schema for describe pathologic events in that arresti-enolase complex. In normal situation, following agonist association/interaction with GPCRs [1–7], and the phosphorylation that ensues [3], arrestin couples with the phosphorylated amino acid residues [3]. As a result the complex of receptor-agonist internalization [4] and then the receptor detaches from arrestin by phosphatase action [4,5] and the freed receptor, along with a fragment of the membrane, returns and fuses with the cell membrane (Fig-6,7] [1,29]. The phosphatase activity may undergo diminution under pathological conditions (A), in which case the epitopes linking the arrestin-enolase couple is externalized (B). The present data suggest that arrestin like proteins can associate with α-enolase and this pair can appear on the surface of the cell membranes as auto antigen (C).

This was tested on rat cardiomyocyte exposed *in vitro* to monoclonal antibodies against arrestin or enolase. Such a treatment resulted in noticeable decrease in cell proliferation, suggesting the presence of arrestin and enolase epitops on membranes of live cells. This phenomenon can be produced by down regulation of phosphatase activity under pathological conditions including cell stress that abolish GPCRs resensitization [27,28].

For describe pathologic events in that arrestin-enolase complex were exposed on the cell membrane, we proposed a modified classic schema [1,29]. In normal situation, following agonist association/interaction with GPCRs (Fig. 4, 1–7), and the phosphorylation that ensues (Fig. 4-3), arrestin couples with the phosphorylated a mino acid residues (Fig. 4-3). As a result the complex of receptor-agonist internalization (Fig. 4-4) and then the receptor detaches from arrestin by phosphatase action (Fig. 4-4,5) and the freed receptor, along with a fragment of the membrane, returns and fuses with the cell membrane (Fig. 4-6, 7). The phosphatase activity may undergo diminution under pathological conditions (Fig. 4A), in which case the epitopes linking the arrestin-enolase couple is externalized (Fig. 4B). The present data suggest that arrestin like proteins can associate with α-enolase and this pair can appear on the surface of the cell membranes as auto antigen (Fig. 4C). This association may be influence the signal transduction by membrane GPCRs as well as the glycolytic pathway in cardiac muscles.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.086.

References

[1] V.V. Gurevich, E.V. Gurevich, Overview of different mechanisms of arrestin-mediated signaling, Curr. Protoc. Pharmacol. 67 (2014) 1–9, http://dx.doi.org/10.1002/0471141755.ph0210s67.
[2] C.E. Alvarez, On the origins of arrestin and rhodopsin, BMC Evol. Biol. 29 (2008) 222–235, http://dx.doi.org/10.1186/1471-2148-8-222.
[3] L. Kristen, P.R.J. Lefkowitz, Classical and new roles of β-arrestins in the regulation of G-PROTEIN-COUPLED receptors, Nat. Rev. Neurosci. 2 (2001) 727–733.
[4] X. Song, S. Coffa, H. Fu, V.V. Gurevich, How does arrestin assemble MAPKs into a signaling complex? J. Biol. Chem. 2 (284) (2009) 685–695, http://dx.doi.org/10.1074/jbc.M806124200.
[5] Y. De Kozak, M. Mirshahi, C. Boucheix, J.P. Faure, Inhibition of experimental autoimmune uveoretinitis in rats by S-antigen-specific monoclonal antibodies, Eur. J. Immunol. 15 (1985) 1107–1111.
[6] R. Seifert, Functional selectivity of G-protein-coupled receptors: from recombinant systems to native human cells, Biochem. Pharmacol. 1 (86) (2013) 853–861, http://dx.doi.org/10.1016/j.bcp.2013.07.029.
[7] R. Lopez-Alemany, M. Suelves, A. Diaz-Ramos, B. Vidal, P. Munoz-Canoves, Alpha-enolase plasminogen receptor in myogenesis, Front. Biosci. 10 (Jan 1 2005) 30–36.
[8] E. Creemers, J. Cleutjens, J. Smits, S. Heymans, L. Moons, D. Collen, M. Daemen, P. Carmeliet, Disruption of the plasminogen gene in mice abolishes wound healing after myocardial infarction, Am. J. Pathol. 156 (2000) 1865–1873.
[9] À. Díaz-Ramos, A. Roig-Borrellas, A. García-Melero, A. Lorens, R. López-Alemany, Requirement of plasminogen binding to its cell-surface receptor α-enolase for efficient regeneration of normal and dystrophic skeletal muscle, PLoS One 7 (12) (2012) e50477, http://dx.doi.org/10.1371/journal.pone.0050477.
[10] M. Li, H. Wen, Z. Yan, T. Ding, L. Long, H. Qin, H. Wang, F. Zhang, Temporal-spatial expression of ENOLASE after acute spinal cord injury in adult rats, Neurosci. Res. 79 (2014) 76–82, http://dx.doi.org/10.1016/j.neures.2013.12.001.
[11] J. Mair, Progress in myocardial damage detection: new biochemical markers for clinicians, Crit. Rev. Clin. Lab. Sci. 34 (1997) 1–66.
[12] M. Mirshahi, L. Camoin, C. Nicolas, I. Ghedira, J. Cozette, J.P. Faure, Copurification of selected glycolytic enzymes with retin al S-antigen (arrestin) by hydroxyapatite agarose chromatography of bovine retina, Curr. Eye Res. 18 (1999) 327–334.
[13] M. Mirshahi, A. Razaghi, R. Stiemer, J.P. Faure, Arrestin-like Protein in Cardiac Muscle, American Heart association, Dallas, USA, 1990.
[14] F. Forooghian, R.K. Cheung, W.C. Smith, P. O'Connor, H.M. Dosch, Enolase and arrestin are novel nonmyelin autoantigens in multiple sclerosis, J. Clin. Immunol. 27 (4) (2007) 388–396.
[15] C. Dorey, J. Cozette, J.P. Faure, A simple and rapid method for isolation of retinal S antigen, Ophthalmic Res. 14 (1982) 249–255.
[16] M. Mirshahi, C. Boucheix, G. Collenot, B. Thillaye, J.P. Faure, Retinal S-antigen epitopes in vertebrate and invertebrate photoreceptors, Invest Ophthalmol. Vis. Sci. 26 (1985) 1016–1021.
[17] R.H. Stiemer, U. Westenfelder, H. Gausepohl, M. Mirshahi, A. Gundt, R.W. Frank, D.N. Männel, A common epitope on human tumor necrosis factor alpha and the autoantigen 'S-antigen/arrestin' induces TNF-alpha production, J. Autoimmun. 5 (1992) 15–26.
[18] M. Mirshahi, F. Borgese, A. Razaghi, U. Scheuring, F. Garcia-Romeu, J.P. Faure, R. Motais, Immunological detection of arrestin, a phototransduction regulatory protein, in the cytosol of nucleated erythrocytes, FEBS Lett. 4 (258) (1989) 240–243.
[19] R. López-Alemany, C. Longstaff, S. Hawley, M. Mirshahi, P. Fábregas, M. Jardí, E. Merton, L.A. Miles, J. Félez, Inhibition of cell surface mediated plasminogen activation by a monoclonal antibody against alpha-Enolase, Am. J. Hematol. 72 (2003) 234–242.
[20] C. Antoine, J.P. Lellouche, J. Maclouf, P. Pradelles, Development of enzyme immunoassays for leukotrienes using acetylcholinesterase, Biochim. Biophys. Acta 10 (1075) (1991) 162–168.
[21] R.H. Aebersold, J. Leavitt, R.A. Saavedra, L.E. Hood, S.B. Kent, Internal amino acid sequence analysis of proteins separated by one- or two-dimensional gel electrophoresis after in situ protease digestion on nitrocellulose, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 6970–6974.
[22] P. Edman, G. Begg, A protein sequencer, Eur. J. Biochem. 1 (1967) 80–91.
[23] M. Baumann, Comparative gas phase and pulsed liquid phase sequencing on a modified applied biosystems 477A sequencer, Anal. Biochem. 1 (190) (1990) 198–208.
[24] R. Lopez-Alemany, P. Correc, L. Camoin, P. Burtin, Purification of the plasmin receptor from human carcinoma cells and comparison to alpha-enolase, Thromb. Res. 15 (75) (1994) 371–381.
[25] E.F. Plow, R. Das, Enolase-1 as a plasminogen receptor, Blood 28 (113) (2009) 5371–5372, http://dx.doi.org/10.1182/blood-2009-03-208546.
[26] J.A. Gerlt, P.C. Babbitt, M.P. Jacobson, S.C. Almo, Divergent evolution in enolase superfamily: strategies for assigning functions, J. Biol. Chem. 2 (287) (2012) 29–34, http://dx.doi.org/10.1074/jbc.R111.240945.
[27] N.T. Vasudevan, M.L. Mohan, M.K. Gupta, A.K. Hussain, S.V. Naga Prasad, Inhibition of protein phosphatase 2A activity by PI3Kγ regulates β-adrenergic receptor function, Mol. Cell. 18 (41) (2011) 636–648, http://dx.doi.org/10.1016/j.molcel.
[28] N.T. Vasudevan, M.L. Mohan, S.K. Goswami, S.V. Naga Prasad, Regulation of β-adrenergic receptor function: an emphasis on receptor resensitization, Cell. Cycle. Nov. 1 (10) (2011) 3684–3691, http://dx.doi.org/10.4161/cc.10.21.18042.
[29] L. Pan, E.V. Gurevich, V.V. Gurevich, The nature of the arrestin-receptor complex determines the ultimate fate of the internalized receptor, J. Biol. Chem. 278 (2003) 11623–11632.