Original Research

Comparative evaluation of CacyBP/SIP protein, β-catenin, and immunoproteasome subunit LMP7 in the heart of rats with hypertension of different etiology

Irena Kasacka[1], Żaneta Piotrowska[1], Jolanta Weresa[2] and Anna Filipek[3]

[1]Department of Histology and Cytophysiology, Medical University of Bialystok, Bialystok 15-222, Poland; [2]Department of Experimental Physiology and Pathophysiology, Medical University of Bialystok, Bialystok 15-222, Poland; [3]Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology, Warsaw 02-093, Poland

Corresponding author: Irena Kasacka. Email: kasacka@umb.edu.pl

Impact statement

Despite extensive research into the pathogenesis of hypertension and disease-related end organ damage, the mechanisms leading to cardiac complications of hypertensive patients are still not fully elucidated. The aim of the presented research was immunodetection and evaluation of CacyBP/SIP, β-catenin, and proteasomes in the hearts of rats with hypertension of different etiology. Our results show an innovative and important network of interactions between proteins potentially involved in the development and progression of heart problems in various types of hypertension. This report might contribute to deeper understanding of the role of the CacyBP/SIP protein, β-catenin, and proteasomes in heart function. Our results might also bring new information concerning the intracellular processes and signal pathways involved in the regulation of cardiomyocytes functioning in hypertension state. In addition to cognitive significance, the results of presented studies may contribute to further successes in preventing and treatment of cardiac complications associated with hypertension.

Abstract

Calcyclin-binding protein/Siah-1-interacting protein (CacyBP/SIP) is the recently discovered peptide, which participates in various intracellular processes. Recent reports indicated that CacyBP/SIP activates the ubiquitin ligases and promotes proteasomal degradation of proteins. One of the most important proteins degraded in CacyBP/SIP-dependent pathway is β-catenin. Considering the key importance of β-catenin in the functioning of the cardiovascular system and in the view of the close relationship between CacyBP/SIP, β-catenin, and proteasomal activity, we have decided to undertake research to identify and evaluate the distribution of CacyBP/SIP, β-catenin and the LMP7 subunit of the immunoproteasome in the heart of rats with hypertension of various etiology. The studies were carried out on the hearts of rats with spontaneous hypertension (SHR), renovascular hypertension, and DOCA-salt hypertension. The myocardial expression of CacyBP/SIP, β-catenin, and LMP7 was detected by immunohistochemistry using the EnVision method. The hypertension significantly increased the immunoreactivity to CacyBP/SIP and LMP-7, while weakening the β-catenin immunoreaction. The intensity of the observed changes depends on the type of hypertension. Our results show an innovative and important network of interactions between proteins potentially involved in the development and progression of heart problems in various types of hypertension. This report might contribute to deeper understanding of the role of the CacyBP/SIP protein, β-catenin, and immunoproteasomes in heart function, as well as to bringing new information concerning pathophysiologic mechanisms leading to cardiac dysfunction in the state of elevated blood pressure.

Keywords: Calcyclin-binding protein/Siah-1-interacting protein, β-Catenin, LMP7, heart, hypertension, immunohistochemistry

Experimental Biology and Medicine **2018; 243: 1199–1206. DOI: 10.1177/1535370218815435**

Introduction

CacyBP/SIP is a protein, which was discovered as a ligand of calcyclin (S100A6) and of Siah-1 (SIP).[1,2] It is now known that CacyBP/SIP has the ability to interact with various intracellular molecules, such as different members of the S100 protein family, i.e. S100A6, S100A1, S100A12, S100B, and S100P, and different proteins forming ubiquitin ligase such as Siah1 and Skp1.[2–4] CacyBP/SIP also attaches to the cytoskeletal proteins, induces actin and microtubules polymerization, and also stabilizes the cytoskeletal organization.[5]

Other disclosed ligands of CacyBP/SIP are the extracellular signal-regulated kinases (ERK1/2). It has been shown

that CacyBP/SIP dephosphorylates ERK1/2, and results in a blockade of the subsequent signaling cascade.[6]

CacyBP/SIP is implicated in a great variety of cellular processes including cell proliferation and differentiation. Research concerning the role of CacyBP/SIP in tumorigenesis has revealed that this protein suppresses the proliferative potential of cancer cells.[7] Several other reports have shown that the CacyBP/SIP gene is upregulated in various differentiating cells, for example in thymocytes, in erythroid cells upon stimulation with erythropoietin, in neurons during brain development, and in endometrial tissue during decidualization process in early pregnancy.[8–11] Increased CacyBP/SIP level has also been found in rat cardiomyocytes during the heart development and in cultured cardiac myoblasts (H9C2 cells) subjected to differentiating conditions.[12] Further investigation on H9C2 cells has revealed that CacyBP/SIP promotes cardiomyocyte differentiation and accelerates formation of myotubes.[12]

The recent literature has highlighted the relationship between CacyBP/SIP and β-catenin, a transcription factor regulating multiple physiological and pathological processes.[4] CacyBP/SIP was found to be a component of an ubiquitin ligase complex responsible for the degradation of β-catenin. Interestingly, it has been proven that β-catenin is of crucial importance for cardiovascular system functioning. *In vitro* studies have demonstrated that β-catenin limits the apoptosis of cardiomyocytes.[13] It has also been shown that β-catenin leads to the activation of inflammatory, hypertrophic, and fibrotic changes in the heart.[14,15] By regulating the β-catenin level, CacyBP/SIP might significantly influence cardiovascular homeostasis. This aspect, however, needs to be clarified by future studies. To the best of our knowledge, there is only one report investigating the possible influence of CacyBP/SIP on cardiac cell viability.[12] It has been demonstrated that CacyBP/SIP reduced the cardiomyocytes injury in the conditions of hypoxia–reoxygenation stress.[12]

Cellular physiology is strictly dependent on the balance between protein synthesis and degradation. Approximately 80–90% of proteins undergo non-lysosomal degradation with the participation of the ubiquitin-proteasome system (UPS). The UPS is responsible for the degradation of short-lived, damaged, non-functional or dispensable proteins in cells. Upon activation of the inflammatory response, constitutive proteasomes are structurally modified into immunoproteasomes, which produce antigenic peptides presented to immune cells. In the immunoproteasomes, the standard subunits β1, β2, and β5 are replaced by so-called immunosubunits β1i (LMP2), β2i (LMP10), β5i (LMP7), respectively.[16] There is overwhelming evidence proving participation of UPS in the development and progression of cardiovascular complications.[17–19] Experimental data have shown enhanced proteasomal activity and production of immunosubunits in the hearts of rodents with Ang II-induced cardiac hypertrophy, experimental hypertension, diabetes, myocardial ischemia, ischemia/reperfusion injury, viral myocarditis, and heart pressure overload.[17–19] It has also been demonstrated that the administration of proteasome inhibitors reduced cardiac injury and heart remodeling associated with hypertension, hyperglycemia, viral myocarditis, doxorubicin-induced cardiomyopathy.[16,20]

Since it has not been examined, whether the state of elevated blood pressure leads to changes in expression of CacyBP/SIP in the heart, and bearing in mind tight associations between CacyBP/SIP, proteasomal activity and β-catenin, we have decided to undertake the research aiming to evaluate the distribution of CacyBP/SIP, β-catenin, and immunoproteasome subunit LMP7 in the hearts of rats with hypertension of different etiology.

Material and methods

Experimental animals

The assumptions, the aim, and the plan of the study, as well as the approach to animals were approved by the local Ethics Committee for Studies on Animal Subjects in Olsztyn.

The study was performed on 24 ($n = 24$) young male Wistar rats, 7 male spontaneously hypertensive rats (SHRs), and 5 normotensive male Wistar Kyoto (WKY) rats. The rats were six weeks of age, and their body weight at the beginning of the experiment was within 170–200 g. The animals were housed at constant humidity ($60 \pm 5\%$) and temperature ($22 \pm 1^{\circ}C$) and were kept under a 12/12-h light/dark cycle. The rats had free access to standard granulated chow and a normal drinking water.

The experimental animals were divided into six groups:

SHR	Seven rats with genetically determined systemic hypertension, inbred strain established from Wistar rats selected for high blood pressure.
Control 1	Five normotensive WKY rats, being the reference for SHR rats
2K1C	Seven Wistar rats with renovascular hypertension induced by the ligation of the artery supplying the blood to the left kidney (two kidney, one clip model of hypertension)
Control 2	Five Wistar rats underwent sham operation (submitted to the same surgical procedure as the hypertensive rats, however, without arterial ligation), being the reference for 2K1C
DOCA-salt	Seven Wistar rats were uninephrectomized, and then rendered hypertensive by high-salt diet and deoxycorticosterone acetate (DOCA) injections
Control 3	Five Wistar rats uninephrectomized only, being the reference for DOCA-salt hypertensive rats

The male SHR, WKY, DOCA-salt hypertensive rats and reference group for rats with DOCA-salt hypertension were housed and purchased from the Department of Experimental Physiology and Pathophysiology, Medical University of Bialystok, Poland.

2K1C renovascular hypertension

Induction of experimental hypertension was performed according to the procedure by Goldblatt *et al.*[21] After the rats were anesthetized by exposure to pentobarbital (40 mg/kg, i.p.), a 3-cm retroperitoneal flank incision was

performed under the sterile conditions. The left kidney was exposed and the renal artery was carefully dissected free of the renal vein. The renal artery was then partially occluded by placing a silver clip with an internal diameter of 0.20 mm on the vessel. The wound was closed with a running 3–0 silk suture ($n = 10$). Sham-operated rats ($n = 5$) underwent identical surgical procedures, except that a clip was not applied to the renal artery. After the surgery, the rats were kept in single cages till wound healing.

DOCA-salt hypertension

Animals were anesthetized by intraperitoneal injection of pentobarbital sodium (300 μmol or ~70 mg/kg of body weight (b.w.)). The right kidney was removed in all rats via a right lateral abdominal incision. After a one-week recovery period, hypertension was induced over a time period of four weeks by s.c. injections of DOCA (67 μmol or ~25 mg/kg in 0.4 ml/kg of dimethylformamide; DMF) twice weekly and replacement of drinking water with 1% NaCl solution. Normotensive control rats were also uninephrectomized but received the vehicle for DOCA (DMF, 0.4 mL/kg, s.c.) twice weekly and drank tap water.

Blood pressure measurement by an indirect method in wakeful rats

After six-week period of the experiment, the systolic BP was measured in all animals by using a noninvasive tail-cuff method (using a Rat Tail Blood Pressure Monitor, Hugo Sachs Elektronik-Harvard Apparatus, March–Hugstetten, Germany). BP measurements were considered valid only when three consecutive readings did not differ by more than 5 mmHg. The average of the three measured values was then recorded. The measurements of BP proved the systolic hypertension in SHR, 2K1C, and DOCA-salt rats (those animals had values of SBP equal to or higher than 150 mmHg).

Method of experimental material collection and fixation

At six week of experiment, the fragments of heart were collected under deep pentobarbital anesthesia (50 mg/kg of body weight) from all rats. The specimens were fixed in 4% buffered formalin for 48 h at 4°C and processed routinely for embedding in paraffin. Sections were cut at 4 μm in thickness and stained by hematoxylin–eosin (H+E) for general histological examination and processed by immunohistochemistry for CacyBP/SIP, β-catenin, LMP7 detection.

Identification of CacyBP/SIP, β-catenin, LMP7 by immunohistochemical methods

In the immunohistochemical study, the EnVision method was used according to Herman and Elfont.[22] Immunostaining was performed by the following protocol. Paraffin-embedded sections were deparaffined and hydrated in pure alcohols. For antigen retrieval, the sections were subjected to pre-treatment in a pressure chamber heated for 1 min at 125°C. During antigen retrieval, sections were incubated with Target Retrieval Solution Citrate pH = 6.0 (S 2369 Dako; Glostrup, Denmark). After cooling down to room temperature, the sections were incubated with Peroxidase Blocking Reagent (S 2001 Dako; Glostrup, Denmark) for 10 min to block endogenous peroxidase activity. Subsequently, the sections were incubated with the primary antibody for CacyBP/SIP and β-catenin (purchased from Nencki Institute of Experimental Biology, produced in-house) and for LMP7 (Enzo Life Sciences BML-PW8845). The antisera were previously diluted in Antibody Diluent (S 0809 Dako Cytomation, Glostrup, Denmark) in relation 1:50 for CacyBP/SIP antibody and in relation 1:5000 for β-catenin antibody and anti-LMP7. Incubation with CacyBP/SIP, β-catenin, and LMP7 antibody lasted overnight and was carried out at 4°C in a humidified chamber. Procedure was followed by incubation with secondary antibody (conjugated to horseradish peroxidase-labeled polymer). The bound antibodies were visualized by 1 min incubation with liquid 3,3′-diaminobenzidine substrate chromogen. The sections were finally counterstained in hematoxylin QS (H – 3404, Vector Laboratories; Burlingame, CA), mounted and evaluated under light microscope. Appropriate washing with Wash Buffer (S 3006 Dako Cytomation; Glostrup, Denmark) was performed between each step. Specificity tests, performed for the CacyBP/SIP, β-catenin, and LMP7 antibody included: negative control, where the primary antibodies were omitted, only antibody diluent was used, and a positive control was prepared with specific tissue as it was recommended by the manufacturer. Histological preparations were subjected to a visual analysis using an Olympus BX41 light microscope with Olympus DP12 digital camera and a PC computer and documented.

Quantitative analysis

The results of immunohistochemical staining were submitted for the evaluation in an Olympus BX41 microscope with Olympus DP12 camera. From each animal, 11 sections of heart were studied (two sections for H+E staining, and three sections for each: CacyBP/SIP, β-catenin and LMP7 immunostaining). From all sections of heart, five randomly selected microscopic fields (each field of 0.785 mm^2, magnification of 200× (20× the lens and 10× the eyepiece)) were documented. Subsequently, images were submitted to morphometric evaluation by using NIS-Elements Advanced Research software of Nikon. In each analyzed images of the heart was measured the intensity of immunohistochemical reaction, using a 0 to 256 grey scale level, where the completely white or bright pixels were scored 0 and completely black pixels were scored 256. Similarly, in each analyzed image of the heart, the width of 25 randomly selected cardiomyocytes was measured and presented as mean values.

All presented data were statistically analyzed by means of software computer package Statistica Version 12.0. The corresponding mean values were computed automatically; significant differences were determined by one-way ANOVA test; $P < 0.05$ was taken as the level of significance.

Results

Systolic hypertension was demonstrated in SHR, 2K1C, and DOCA-salt rats. The mean values of BP for the each studied group are presented in Table 1.

Table 1. Mean values of systolic blood pressure (mmHg) of rats in control and hypertensive groups (mean ± SD).

Model of hypertension	Values of BP (mmHg)	
	Control group	Hypertensive group
SHR	122.3 ± 2.3	160.8 ± 3.3*
2K1C	120.2 ± 2.2	162.6 ± 2.2*
DOCA-salt	126.0 ± 4.0	180.0 ± 13.0*

*$P < 0.005$ control group vs. hypertensive group.
SHR: spontaneously hypertensive rat; DOCA: deoxycorticosterone acetate.

The H&E staining demonstrated the histopathologic changes in the heart of hypertensive rats such as hypertrophy of cardiomyocytes, and influx in the number of immune cells when compared to normotensive animals (Figure 1(a) to (d)). Wherein, the greatest abundance of inflow cells has been stated in the heart of SHR rats (Figure 1(b)). The following morphometric analysis confirmed an increase in the width of cardiomyocytes in all rat models of hypertension in relation to control rats. The most severe cardiac cell hypertrophy was observed in SHR rats, while in DOCA-salt hypertensive rats, the cardiomyocytes overgrowth was the mildest (Table 2).

Positive immunohistochemical reaction for CacyBP/SIP, β-catenin, and LMP7 has been noted in the hearts of all studied rats, except for negative LMP7 immunolabeling in the heart of normotensive animals. The density and intensity of CacyBP/SIP-, β-catenin- and LMP7-immunostaining varied between the experimental and control groups.

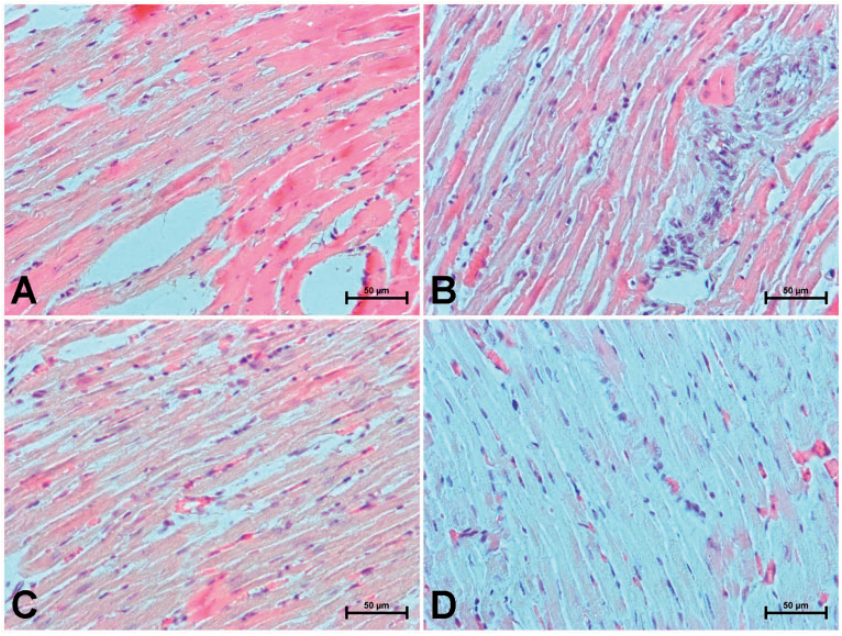

Figure 1. The heart section of control rat (a); rat with spontaneous hypertension (b); 2K1C rat (c) and DOCA-salt hypertensive rat (d) H & E staining. (A color version of this figure is available in the online journal.)

Table 2. Width of cardiomyocytes (μm) and intensity of immunoreaction against CacyBP/SIP, β-catenin, and LMP7 in the heart of rats subjected to different models of hypertension (mean ± SE).

Group of rats	Width of cardiomyocytes (μm)	Intensity of immunohistochemical reaction Scale from 0 (white pixel) to 256 (black pixel)		
		CacyBP/SIP	β-catenin	LMP7
Control	7.7 ± 0.17	74.9 ± 2.37	148.4 ± 4.46	Not detectable
SHR	13.1 ± 0.24^{a}	96.2 ± 3.00$^{a\uparrow}$	126.4 ± 3.55$^{a\downarrow}$	109.4 ± 3.27$^{a\uparrow}$
2K1C	10.2 ± 0.21^{a,b}	85.7 ± 2.16$^{a\uparrow,b\downarrow}$	88.4 ± 2.36$^{a\downarrow,b\downarrow}$	93.7 ± 1.74$^{a\uparrow,b\downarrow}$
DOCA-salt	9.2 ± 0.17^{a,c,d}	130.4 ± 3.25$^{a\uparrow,c\uparrow,d\uparrow}$	114.5 ± 3.44$^{a\downarrow,c\downarrow,d\uparrow}$	90.3 ± 1.98$^{a\uparrow,c\downarrow}$

[a]$P < 0.05$ hypertensive vs. normotensive.
[b]$P < 0.05$ 2K1C vs. SHR.
[c]$P < 0.05$ DOCA-salt vs. SHR.
[d]$P < 0.05$ DOCA-salt vs. 2K1C.
$^{\downarrow}$Weakening of immunohistochemical reaction.
$^{\uparrow}$Intensification of immunohistochemical reaction.
SHR: spontaneously hypertensive rat; DOCA: deoxycorticosterone acetate.

CacyBP/SIP-immunoreactivity occurred in the form of small brown-stained granules diffusely distributed in the cytoplasm of cardiomyocytes (Figure 2(a) to (d)). Immunodetection of CacyBP/SIP in the heart of normotensive rats gave weak reaction in cardiac muscle cells (Figure 2 (a)). Considerable greater intensity of the CacyBP/SIP-immunosignal was found in the heart of all hypertensive groups compared to control rats (Figure 2(b) to (d)). In the heart of 2K1C, the CacyBP/SIP immunoreactivity was increased in the slightest extent, whereas in DOCA-salt hypertensive rats, the result of reaction with anti-CacyBP/SIP antibody was the strongest in comparison to other studied experimental and control groups (Figure 2(d)).

The immunohistochemical studies have revealed that β-catenin is redispositionally located in intercalated discs (ICDs) (Figure 3(a) to (d)). In the heart of control rats was noted strong β-catenin immunoreactivity in ICDs (Figure 3 (a)). The β-catenin immunosignal was markedly lessen in the hearts of SHR, 2K1C, and DOCA-salt hypertensive rats than in normotensive animals (Figure 3(b) to (d)).

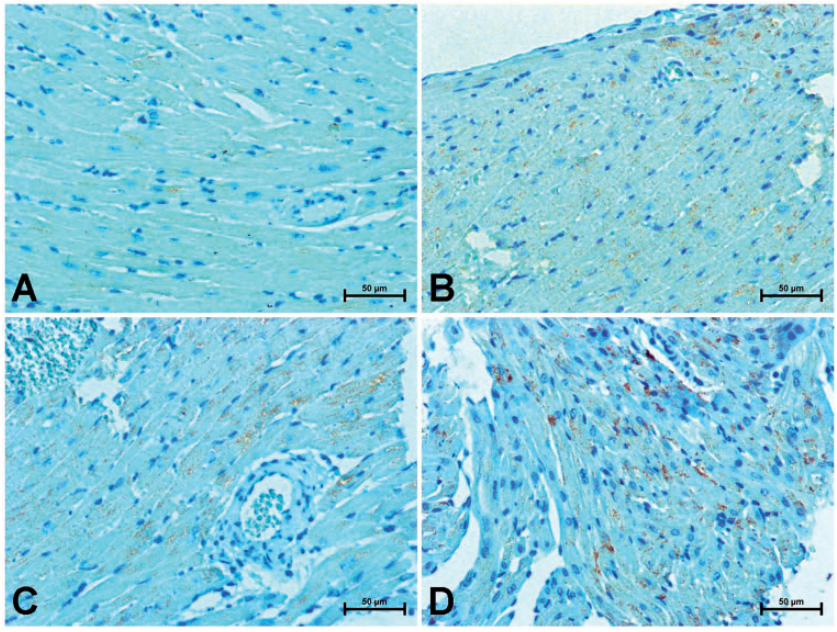

Figure 2. Immunodetection of CacyBP/SIP protein in the heart of normotensive rat (a); SHR rat (b); rat with renovascular hypertension (c) and DOCA-salt hypertensive rat (d). (A color version of this figure is available in the online journal.)

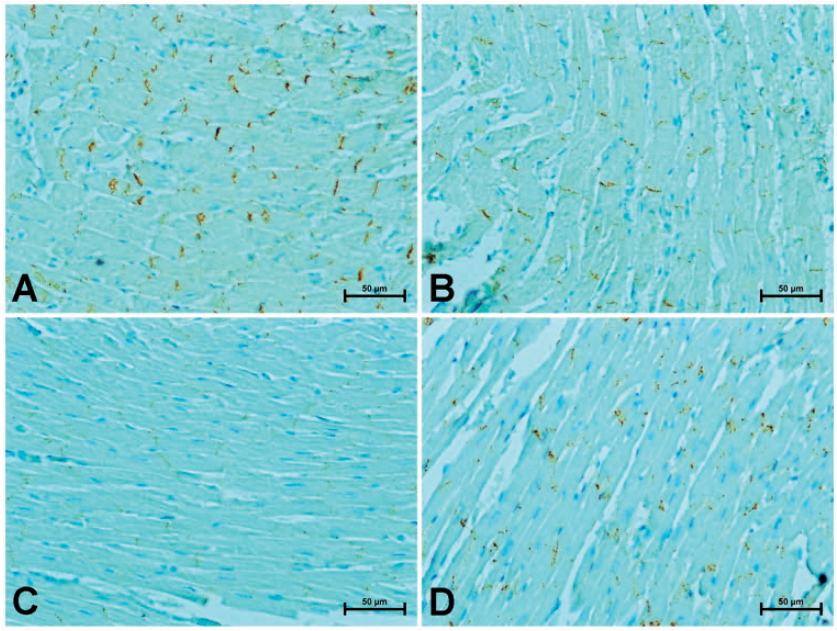

Figure 3. Immunohistochemical reaction determining β-catenin in the heart of control rat (a); at with spontaneous hypertension (b); 2K1C rat (c) and DOCA-salt hypertensive rat (d). (A color version of this figure is available in the online journal.)

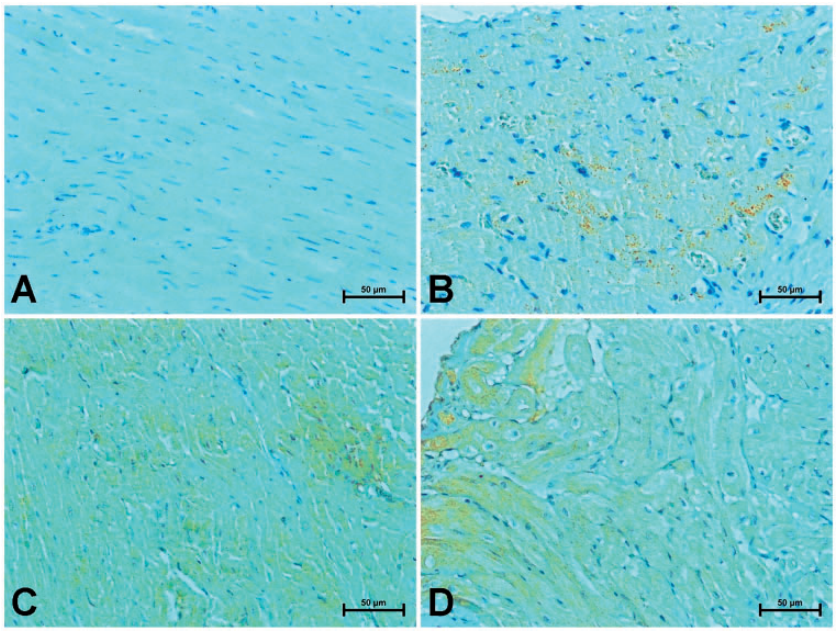

Figure 4. Representative immunohistochemical photomicrographs of LMP7 in the heart of normotensive rat (a); SHR rat (b); rat with renovascular hypertension (c) and DOCA-salt hypertensive rat (d). (A color version of this figure is available in the online journal.)

Relatively, the smallest decrease in β-catenin immunoreactivity was seen in the SHR rats (Figure 3(b)), in turn, the intensity of β-catenin immunostaining was the lowest in 2K1C rats when compared to other studied hypertensive groups and normotensive animals (Figure 3(c)).

The antisera against LMP-7 did not reveal the presence of immunosubunit in the heart of normotensive animals (Figure 4(a)), while the noticeable LMP-7 immunoreactivity was found in the heart of the studied experimental groups (Figure 4(b) to (d)). It is worth highlighting that in the heart of SHR rats, the intensity of LMP-7 immunoreaction was the strongest in relation to the remaining hypertensive groups (Figure 4(b)).

The visually perceived differences in the intensity of the immunohistochemical reaction against CacyBP/SIP, β-catenin, and LMP7 in the hearts of control and hypertensive rats have been confirmed by the computer image analysis (Table 2).

Discussion

Currently, we have been evaluating and comparing the distribution of CacyBP/SIP, β-catenin, and LMP7 subunit in the heart of rats with spontaneous, renovascular, and DOCA-salt hypertension. Our research has revealed that the biosynthesis of CacyBP/SIP, β-catenin, and LMP7 in the rat heart is altered in the state of elevated blood pressure. However, the intensity of changes in CacyBP/SIP, β-catenin, and LMP7 content in the heart was dependent on disease etiology.

In the present study, we have demonstrated the increased intensity of immunoreaction determining CacyBP/SIP in the hearts of hypertensive rats compared to normotensive animals, with the strongest CacyBP/SIP-immunostaining in rats with DOCA-salt hypertension.

CacyBP/SIP is the multi-domain protein, having the ability to interact with various intracellular targets.[4] The latest reports have revealed that CacyBP/SIP binds to cytoskeletal proteins such as tubulin, tropomyosin, actin, intensifies the polymerization of microtubules and microfilaments, as well as stabilizes the cytoskeletal organization.[4,5]

Moreover, it has been stated that CacyBP/SIP restricts the interaction between actin and myosin and inhibits the activity of actin-activated myosin ATPase.[4] Based upon our data, increased CacyBP/SIP content in the heart of hypertensive rats might be potentially responsible for worsening the cardiomyocytes contractility in the state of elevated blood pressure. However, the current state of knowledge about the importance of CacyBP/SIP in the cardiovascular system is still far from sufficient. In available literature, there is only one report investigating the possible influence of CacyBP/SIP on cardiac cell differentiation and viability.[12]

The literature data indicated the greatest impairment of heart systolic and diastolic function in DOCA-salt hypertensive rats when compared to other experimental models of hypertension.[23] We reported herein the most substantial elevation of CacyBP/SIP level in rats with DOCA-salt hypertension compared to SHR and 2K1C hypertensive rats. Considering inhibitory influence of CacyBP/SIP on actin–myosin interplay, this finding might constitute a possible explanation of most severe cardiac dysfunction in this hypertension model.

Current paper has confirmed that β-catenin is predominantly located in the intercalated discs of cardiomyocytes. Our report has also showed decreased β-catenin immunoreactivity in the hearts of all studied hypertensive groups in comparison to control animals. Presented results are

consistent with the report by Zheng *et al.*,[24] who demonstrated the lowered β-catenin immunosignal within the intercalated discs in the hearts of SHRs. Wang and Gerdes[25] and Yoshida *et al.*[26] found similar decline of β-catenin level in the cardiac tissue of guinea pigs and hamsters with heart failure.

There are two known pathways for proteasomal degradation of β-catenin. The first, classical pathway is mediated by ligase complex $SCF^{\beta TrCP}$ and results in degradation of phosphorylated β-catenin, whereas in the second, recently discovered pathway, the CacyBP/SIP protein participates. CacyBP/SIP forms a complex with Siah-1 and Skp1 ligases, called SCF^{TBL1}, and leads to the degradation of non-phosphorylated β-catenin.[4] Given the elevated CacyBP/SIP content in cardiac tissue of SHR, 2K1C, and DOCA-salt hypertensive rats, demonstrated in presented report, it might be expected that the reduction of β-catenin content in heart in the state of elevated blood pressure might be associated with CacyBP/SIP-determined protein degradation.

The overwhelming body of evidence points to the involvement of β-catenin in the cardiomyocyte hypertrophy. Haq *et al.*[14] demonstrated that the modification of β-catenin gene resulting in blocked degradation and accumulation of β-catenin provokes the enlargement of cultured neonatal rat ventricular myocytes (NRVMs).[14] In accordance, Zhang *et al.*[27] indicated that the inactivation of β-catenin gene attenuated the overgrowth of NRVMs evoked by the treatment with hypertrophic stimuli. Further in vivo studies showed that mice subjected to genetically β-catenin stabilization developed heart hypertrophy compared to not modified animals.[14] In longer lasting experiment, Hirschy *et al.*[28] evidenced that the inactivation of β-catenin gene leaded to adaptive heart hypertrophy, which was well tolerated by mice. In turn, β-catenin stabilization led to dilated cardiomyopathy and mice death by five months of age.[28] Other report by Zelarayán *et al.*[29]showed that the genetic β-catenin depletion significantly improved the survival and left ventricular function in mice undergoing myocardial infarction, while genetic β-catenin stabilization had opposite effects. In this manner, as observed in this study, decreased β-catenin amount in the heart of rats with hypertension might be interpreted as adaptive mechanism aimed to minimizing hypertension-evoked cardiac complication.

Our research has revealed the upregulation of LMP7 subunit in the heart of SHR-, 2K1C-, DOCA-salt hypertensive rats, with the strongest increase in LMP7 subunit level in rats with spontaneous hypertension. Those findings are in agreement with the results by Li *et al.*[17] and Yan *et al.*[18] who demonstrated the overexpression of immunoproteasome subunits in cardiac tissue of mice with DOCA-salt hypertension and mice with Ang II-induced heart hypertrophy.

It is well-described that the hypertension is associated with the stimulation of inflammatory processes.[30] In hypertension state, the level of proinflammatory cytokines as C-reactive protein (CRP), interleukines (IL-1 and IL-6), tumor necrosis factor α (TNF-α) is significantly elevated.[30] Various experimental data indicate the infiltration of immune cells (macrophages and lymphocytes) in kidney, vasculature, and heart of hypertensive rodents.[30] In performed routine, histological examination of heart specimens from rats with SHR, 2K1C, and DOCA-salt hypertension, we have noted the greatest infux of immune cells in SHRs compared to other hypertension models. On this basis, it might be supposed that the SHR model of hypertension proceeds with more severe immune response than 2K1C and DOCA-salt hypertension models. As activation of inflammatory processes leads to structural modification of proteasomes into immunoproteasomes our data showing the highest LMP7-immunoreactivity in hearts of SHR rats may be linked to the observed infiltration of inflammatory cells.

The UPS system might play a crucial role in hypertension-evoked cardiac remodelling.[16] During cardiac remodeling, existing sarcomeric proteins are degraded by proteasomes, for building up new ones in heart muscle cell.[16] The biochemical studies revealed that the immunoproteasomes have greater chymotrypsin-like catalytic activity than the constitutively expressed proteasomes, due to LMP7-induced structural change.[31] Therefore, noted in the current report upregulation of LMP7 immunosubunits in the heart of hypertensive rats might be related to increased process of reconstruction of contractile fibers in cardiomyocytes.

Conducted morphometric analysis demonstrated the hypertrophy of cardiomyocytes in all hypertensive rats studied. The most substantial increase in cardiomyocytes width was noted in SHR rats, whereas the cardiac cells overgrowth was the mildest in DOCA-salt hypertensive rats.

The overactivity of renin-angiotensin (RA) system and enhanced generation of Ang II is considered a principle mechanism participating in the development of heart hypertrophy.[32] The Ang II acting through angiotensin II receptor type 1 (AT_1 receptor) leads to changes in the cardiac cell phenotype characterized by the enhanced expression of fetal-type genes, such as skeletal α-actin (skACT) and ß-myosin heavy chain (ß-MHC).[32] Experimental models of hypertension, which was used in presented research, differ in terms of status of the RA system The spontaneous and renovascular hypertension is characterized by the enhanced activity of RA system, while in the DOCA-salt hypertensive rats, the activity of RA system is significantly decreased.[33] Perhaps, reported in the current study, the mildest enlargement of cardiac muscle cells in DOCA-salt hypertensive rats, compared to other model of hypertension, might result from the lack of Ang II-evoked hypertrophic stimulation on cardiomyocytes.

This report might contribute to a deeper understanding of the role of the CacyBP/SIP protein, β-catenin, and immunoproteasomes in the heart function as it provides new insights concerning the pathophysiologic mechanisms leading to cardiac dysfunction in the state of elevated blood pressure.

Authors' contributions: Irena Kasacka, Zaneta Piotrowska and Anna Filipek conceived of and designed the experiments. Irena Kasacka, Joanna Weresa participated in conducting of the experiment. Irena Kasacka, Anna Filipek contributed reagents/materials/analysis tools. Irena Kasacka and Zaneta Piotrowska analyzed the data. Writing – original draft

preparation: Zaneta Piotrowska. Writing – review and editing: Irena Kasacka. Approval of final manuscript: All Authors.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

This work was supported by statutory funds from the Medical University of Bialystok N/ST/ZB/18/002/2232.

REFERENCES

1. Filipek A, Kuźnicki J. Molecular cloning and expression of a mouse brain cDNA encoding a novel protein target of calcyclin. *J Neurochem* 1998;**70**:1793–8
2. Matsuzawa SI, Reed JC. Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses. *Mol Cell*. 2001;**7**:915–26
3. Filipek A, Jastrzebska B, Nowotny M, Kuznicki J. CacyBP/SIP, a calcyclin and Siah-1-interacting protein, binds EF-hand proteins of the S100 family. *J Biol Chem* 2002;**277**:28848–52
4. Topolska-Woś AM, Chazin WJ, Filipek A. CacyBP/SIP-Structure and variety of functions. *Biochim Biophys Acta* 2016;**1860**:79–85
5. Jurewicz E, Ostrowska Z, Jozwiak J, Redowicz MJ, Lesniak W, Moraczewska J, Filipek A. CacyBP/SIP as a novel modulator of the thin filament. *Biochim Biophys Acta* 2013;**1833**:761–6
6. Rosińska S, Leśniak W, Filipek A. Distinct effect of CacyBP/SIP on the ERK1/2-CREB-BDNF pathway in undifferentiated and differentiated neuroblastoma NB2a cells. *Neurochem Int* 2016;**97**:65–72
7. Kilanczyk E, Wasik U, Filipek A. CacyBP/SIP phosphatase activity in neuroblastoma NB2a and colon cancer HCT116 cells. *Biochem Cell Biol* 2012;**90**:558–64
8. Fukushima T, Zapata JM, Singha NC, Thomas M, Kress CL, Krajewska M, Krajewski S, Ronai Z, Reed JC, Matsuzawa S. Critical function for SIP, a ubiquitin E3 ligase component of the beta-catenin degradation pathway, for thymocyte development and G1 checkpoint. *Immunity* 2006;**24**:29–39
9. Jastrzebska B, Filipek A, Nowicka D, Kaczmarek L, Kuznicki J. Calcyclin (S100A6) binding protein (CacyBP) is highly expressed in brain neurons. *J Histochem Cytochem* 2000;**48**:1195–202
10. Pircher TJ, Geiger JN, Zhang D, Miller CP, Gaines P, Wojchowski DM. Integrative signaling by minimal erythropoietin receptor forms and c-Kit. *J Biol Chem* 2001;**276**:8995–9002
11. Yang YJ, Liu WM, Zhou JX, Cao YJ, Li J, Peng S, Wang L, Yuan JG, Duan EK. Expression and hormonal regulation of calcyclin-binding protein (CacyBP) in the mouse uterus during early pregnancy. *Life Sci* 2006;**78**:753–60
12. Au KW, Kou CY, Woo AY, Chim SS, Fung KP, Cheng CH, Waye MM, Tsui SK. Calcyclin binding protein promotes DNA synthesis and differentiation in rat neonatal cardiomyocytes. *J Cell Biochem* 2006;**98**:555–66
13. Hahn JY, Cho HJ, Bae JW, Yuk HS, Kim KI, Park KW, Koo BK, Chae IH, Shin CS, Oh BH, Choi YS, Park YB, Kim HS. Beta-catenin overexpression reduces myocardial infarct size through differential effects on cardiomyocytes and cardiac fibroblasts. *J Biol Chem* 2006;**281**:30979–89
14. Haq S, Michael A, Andreucci M, Bhattacharya K, Dotto P, Walters B, Woodgett J, Kilter H, Force T. Stabilization of beta-catenin by a Wnt-independent mechanism regulates cardiomyocyte growth. *Proc Natl Acad Sci U S A* 2003;**100**:4610–5
15. Lin JC, Chang RL, Chen YF, Yang JJ, Baskaran R, Chung LC, Chen RJ, Day CH, Vijaya Padma V, Huang CY. β-Catenin overexpression causes an increase in inflammatory cytokines and NF-κB activation in cardiomyocytes. *Cell Mol Biol* 2016;**63**:17–22
16. Powell SR. The ubiquitin-proteasome system in cardiac physiology and pathology. *Am J Physiol Heart Circ Physiol* 2006;**291**:1–19
17. Li N, Wang HX, Han QY, Li WJ, Zhang YL, Du J, Xia YL, Li HH. Activation of the cardiac proteasome promotes angiotension II-induced hypertrophy by down-regulation of ATRAP. *J Mol Cell Cardiol* 2015;**79**:303–14
18. Yan W, Bi HL, Liu LX, Li NN, Liu Y, Du J, Wang HX, Li HH. Knockout of immunoproteasome subunit β2i ameliorates cardiac fibrosis and inflammation in DOCA/Salt hypertensive mice. *Biochem Biophys Res Commun* 2017;**490**:84–90
19. Zu L, Bedja D, Fox-Talbot K, Gabrielson KL, Van Kaer L, Becker LC, Cai ZP. Evidence for a role of immunoproteasomes in regulating cardiac muscle mass in diabetic mice. *J Mol Cell Cardiol* 2010;**49**:5–15
20. Yu X, Patterson E, Kem DC. Targeting proteasomes for cardioprotection. *Curr Opin Pharmacol* 2009;**9**:167–72
21. Goldblatt H, Lynch J, Hanzal RF, Summerville WW. Studies on experimental hypertension: I. The production of persistent elevation of systolic blood pressure by means of renal ischemia. *J Exp Med* 1934;**59**:347–79
22. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. *Biotech Histochem* 1991;**66**:194–9
23. Rao MR. Effects of tetrandrine on cardiac and vascular remodeling. *Acta Pharmacol Sin* 2002;**23**:1075–85
24. Zheng Q, Chen P, Xu Z, Li F, Yi XP. Expression and redistribution of β-catenin in the cardiac myocytes of left ventricle of spontaneously hypertensive rat. *J Mol Hist* 2013;**44**:565–73
25. Wang X, Gerdes AM. Chronic pressure overload cardiac hypertrophy and failure in guinea pigs: III. Intercalated disc remodeling. *J Mol Cell Cardiol* 1999;**31**:333–43
26. Yoshida M, Ohkusa T, Nakashima T, Takanari H, Yano M, Takemura G, Honjo H, Kodama I, Mizukami Y, Matsuzaki M. Alterations in adhesion junction precede gap junction remodelling during the development of heart failure in cardiomyopathic hamsters. *Cardiovasc Res* 2011;**92**:95–105
27. Zhang CG, Jia ZQ, Li BH, Zhang H, Liu YN, Chen P, Ma KT, Zhou CY. Beta-Catenin/TCF/LEF1 can directly regulate phenylephrine-induced cell hypertrophy and Anf transcription in cardiomyocytes. *Biochem Biophys Res Commun* 2009;**390**:258–62
28. Hirschy A, Croquelois A, Perriard E, Schoenauer R, Agarkova I, Hoerstrup SP, Taketo MM, Pedrazzini T, Perriard JC, Ehler E. Stabilised beta-catenin in postnatal ventricular myocardium leads to dilated cardiomyopathy and premature death. *Basic Res Cardiol* 2010;**105**:597–608
29. Zelarayán LC, Noack C, Sekkali B, Kmecova J, Gehrke C, Renger A, Zafiriou MP, van der Nagel R, Dietz R, de Windt LJ, Balligand JL, Bergmann MW. Beta-Catenin downregulation attenuates ischemic cardiac remodeling through enhanced resident precursor cell differentiation. *Proc Natl Acad Sci U S A* 2008;**105**:19762–7
30. Li JJ, Fang CH, Hui RT. Is hypertension an inflammatory disease? *Med Hypotheses* 2005;**64**:236–40
31. Ebstein F, Voigt A, Lange N, Warnatsch A, Schröter F, Prozorovski T, Kuckelkorn U, Aktas O, Seifert U, Kloetzel PM, Krüger E. Immunoproteasomes are important for proteostasis in immune responses. *Cell* 2013;**152**:935–7
32. Sadoshima J, Izumo S. Molecular characterization of angiotensin II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT1 receptor subtype. *Circ Res* 1993;**73**:413–23
33. Clozel JP, Müller RK, Roux S, Fischli W, Baumgartner HR. Influence of the status of the renin-angiotensin system on the effect of cilazapril on neointima formation after vascular injury in rats. *Circulation* 1993;**88**:1222–7

(Received July 31, 2018, Accepted November 1, 2018)