Regulatory Peptides 179 (2012) 43–49

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

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

Contribution of caveolin-1 to ventricular nitric oxide in age-related adaptation to hypovolemic state

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ARTICLE INFO

Article history:
Received 24 July 2011
Received in revised form 24 June 2012
Accepted 27 August 2012
Available online 3 September 2012

Keywords:
Caveolin-1
Nitric oxide
Hemorrhage
Aging

ABSTRACT

Our previous results have shown that hypovolemic state induced by acute hemorrhage in young anesthetized rats triggers heterogeneous and dynamic nitric oxide synthase (NOS) activation, modulating the cardiovascular response. Involvement of the nitric oxide pathway is both isoform-specific and time-dependent. The aim of the present study was to investigate changes in activity and protein levels of the different NOS forms, changes in the abundance of caveolin-1 during hypovolemic state and caveolin-1/eNOS association using young and middle-aged rats. Therefore, we studied (i) changes in NOS activity and protein levels and (ii) caveolin-1 abundance, as well as its association with endothelial NOS (eNOS) in ventricles from young and middle-aged rats during hypovolemic state. We used 2-month (young) and 12-month (middle-aged) old male Sprague–Dawley rats. Animals were divided into two groups (n = 14/group): (a) sham; (b) hemorrhaged animals (20% blood loss). With advancing age, we observed an increase in ventricle NOS activity accompanied by a decrease in eNOS and caveolin-1 protein levels, but increased inducible NOS (iNOS). We also observed that aging is associated with caveolin-1 dissociation from eNOS. Myocardia from young and middle-aged rats subjected to hemorrhage-induced hypovolemia exhibited an increase in NOS activity and protein levels with a reduction in caveolin-1 abundance, accompanied by a greater dissociation between eNOS and its regulatory protein. Further, an increase in iNOS protein levels after blood loss was observed only in middle-aged rats. Our evidence suggests that aging and acute hemorrhage contribute to the development of upregulation in NOS activity. Our findings demonstrate that specific expression patterns of ventricular NOS isoforms, alterations in the amount of caveolin-1 and caveolin-1/eNOS interaction are involved in aged-related adjustment to hypovolemic state.

1. Introduction

Circulatory homeostasis is dependent on intravascular volume and distribution. Hypovolemic state can be due to primary intravascular volume loss (hemorrhage), secondary intravascular volume loss (diarrhea, regurgitation/anacatharsis), or increased unstressed vascular volume (loss of sympathetic tone, vasodilating drugs) [1]. Depending on the degree and duration of tissue hypoperfusion, cellular dysfunction, organ injury and death may occur [2]. Acute hemorrhage leads to a well characterized sequence of events that include (i) a decrease in cardiac output and blood pressure, (ii) subsequent release of endogenous vasoconstrictors in an attempt to maintain normal blood pressure, and (iii) delayed vascular decompensation [3]. The formation of NO is one of the pathways that have received significant attention in earlier studies on its involvement in hypovolemic state [4,5]. NO has been shown to participate in a wide range of physiological and pathophysiological cardiovascular processes, including the effect on cardiac myocyte function [6,7]. The role of NO may change significantly in the diseased heart where the expression, activity and/or coupling of NOS isoforms may be altered [8]. Studies have shown that non-selective inhibition of NOS in early hemorrhage increases tissue injury [9], indicating that at least a basal production rate of NO is required to preserve tissue perfusion during this early timeframe. Alterations in NO production with aging have been studied but some of the existing studies have yielded conflicting results regarding cardiac tissue [10,11]. A progressive decline in baseline cardiac function from young (2–4 months) to middle-aged (12–15 months) and aged (24 months) rats has been observed [12–15]. Thus, it would not be surprising if changes in production and/or activity of aging mediators would either promote or delay the disease process.

Moreover, it has been reported that eNOS is quantitatively associated with caveolin, the structural protein of caveolae, in ventricular myocytes and endothelial cells [16]. In cardiac myocytes, eNOS is predominantly associated with caveolin-3 and, to a lesser extent, with caveolin-1. In endothelial cells, in which caveolin-3 is not found, eNOS is quantitatively and specifically associated with caveolin-1 [17]. In addition, García Gardena et al. [18] have reported that the direct binding of eNOS to caveolin-1, per se, and the functional consequences of eNOS targeting to caveolae are probably temporarily and spatially distinct events that regulate NOS activity. Moreover, Cho et

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http://dx.doi.org/10.1016/j.regpep.2012.08.002

al. [19] have suggested that caveolin-1, as a major determinant of the aging process, would be a prime candidate for such role. We have previously shown that hypovolemic state induced by acute hemorrhage in young anesthetized rats triggered heterogeneous and dynamic NOS activation, modulating the cardiovascular response. Involvement of the NO pathway occurs in an isoform-specific and time-dependent manner [4]. Animal studies also suggest that, during the event of shock, NO overproduction from iNOS may contribute to vascular impairment [20]. Nevertheless, an association between caveolin-1 abundance and the ventricular NO system during hypovolemic state and the aging process still remains to be determined. On the basis of published results, the aim of the present study was to investigate changes in activity and protein levels of the different NOS forms and the abundance of caveolin-1 during hypovolemic state, as well as caveolin-1/eNOS association using young and middle-aged rats.

2. Materials and methods

The experiments were performed using male Sprague–Dawley rats aged 2 months (young) and 12 months (middle-aged). The rats were housed in separate cages in a temperature-controlled room with a 12-hour light/dark cycle. They were fed with standard rat chow and tap water ad libitum. Animals were cared for according to the American Physiological Society's Guiding Principles in the Care and Use of Animals and Regulation 6344/96 of Argentina's National Drug, Food and Medical Technology Administration (ANMAT). Experimental procedures were approved by the ethics committee of the School of Biochemistry and Pharmacy (CEFFB), Universidad de Buenos Aires, Argentina.

2.1. Animal preparation

All rats were anesthetized with urethane (1.0 g/kg, ip) and kept under anesthesia by additional small doses of urethane throughout the experiment. The rats were placed on a thermostatically controlled heated table. Body temperature was monitored with a rectal thermometer and maintained between 36 and 38 °C. A tracheotomy was performed using polyethylene (PE-240) tubing to ensure an open airway. Mean arterial pressure (MAP) was measured through a cannula inserted in the right femoral artery connected to a pressure transducer (Statham P23 ID, Gould Inst. Cleveland, OH, USA) and recorded with a polygraph (Physiograph E & M Co, Houston, TX, USA). Heart rate (HR) was derived from the pulsatile pressure signal by beat-to-beat conversion with a tachograph preamplifier (S77-26 tachometer, Coulbourn Inst., Allentown, PA, USA). The Labtech Notebook program (Laboratory Tech., Wilmington, MD, USA) was used for data acquisition. MAP was continuously recorded throughout the experiment. The left femoral artery was cannulated to facilitate blood withdrawal. After a 35-minute equilibrium period, rats were bled at a constant rate for 2 min via a catheter using a syringe. Hemorrhage (20% of the volemia) was induced by drawing blood (1.4 mL/100 g body weight). The total amount of blood drawn was kept constant (total blood volume was 7.0 ± 0.1 mL/100 g body weight).

2.2. Experimental protocols

Two experimental groups were used in the study.

1. Sham rats (S). After a 30-minute stabilization period, basal values of MAP and HR were measured for 5 min. Thereafter, MAP and HR were continuously recorded for 60 min (n = 14).
2. Hemorrhaged rats (H). After a 30-minute stabilization period, control values of MAP and HR were measured for 5 min and then rats were subjected to arterial hemorrhage. Thereafter, MAP and HR were continuously recorded for 60 min (n = 14).

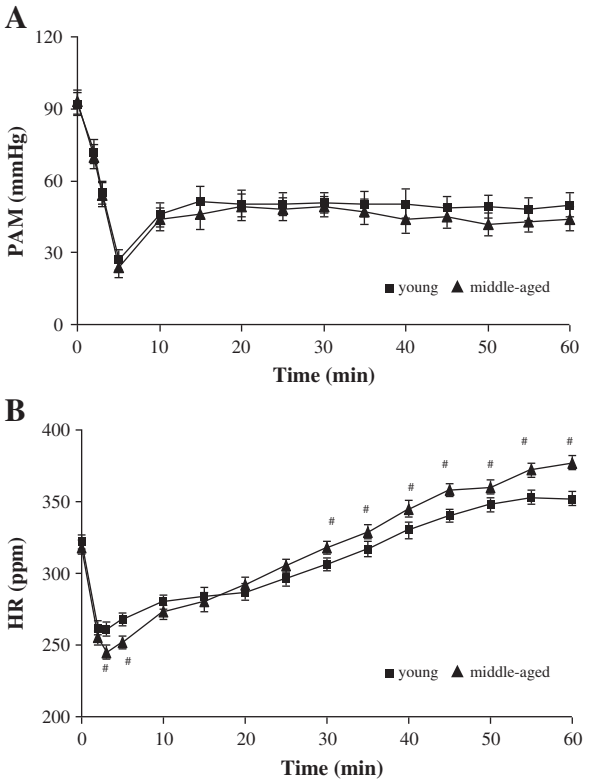

Fig. 1. Changes in mean arterial pressure (A) and heart rate (B) during and after the hemorrhagic state in the H group of young and middle-aged rats. ■ Young animals; ▲ middle-aged animals. Points are mean values (error bars represent SEM) #$P<0.01$ vs. middle-aged H group.

2.3. Nitric oxide synthase activity

NOS activity was determined in the left ventricle from S and H animals 60 min after the beginning of the experiment by measuring the conversion of [14C(U)]-L-arginine to [14C(U)]-L-citrulline. Tissue homogenates (approximately 50 μg protein) were incubated in a Tris–HCl buffer (pH 7.4) containing 1 μg/mL L-arginine, [14C(U)]-L-arginine (346 μCi/mL), L-valine (67 mM), NADPH (1 mM), calmodulin (30 nM), tetrahydrobiopterin (5 μM), and $CaCl_2$ (2 mM) for 60 min at room temperature. The reaction was stopped by adding 175 μL of ice-cold HEPES buffer (pH 5.5) containing EDTA (2 mM) and then applied to AG 50W cation exchange resin columns. The amount of eluted [14C(U)]-L-citrulline was quantified using a liquid scintillation counter (Wallac 1414 WinSpectral; EG&G Company, Turku, Finland) as described previously [21]. All compounds were purchased from Sigma Chemical Co. (Saint Louis, MO, USA) except [U-14C]-L-arginine monohydrochloride (346 mCi/mmol, Wallac 1414 WinSpectral; EG&G Company, Turku, Finland). Protein determination was made using a dye-binding assay (Bio-Rad, Munich, Germany) with bovine serum albumin as standard.

2.4. Western blot analysis

In order to determine the effect of hypovolemic state on nNOS, eNOS, iNOS and caveolin-1 protein levels, fresh left ventricles were isolated from groups S and H (n = 4 each group each time). Rats of both groups were sacrificed 95 min after the surgery and thus rats were studied at 60 min after bleeding. The tissue samples were homogenized on ice with a Tissue Tearor (Biospec Products Inc., Bartlesville, OK, USA) in a homogenization buffer (50 mmol/L Tris, 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 1% Triton, 1 mmol/L PMSF, 1 μmol/L pepstatin, 2 μmol/L leupeptin) and 1× protease inhibitor cocktail from Roche Diagnostics. Protein concentration in the Triton-soluble supernatant was

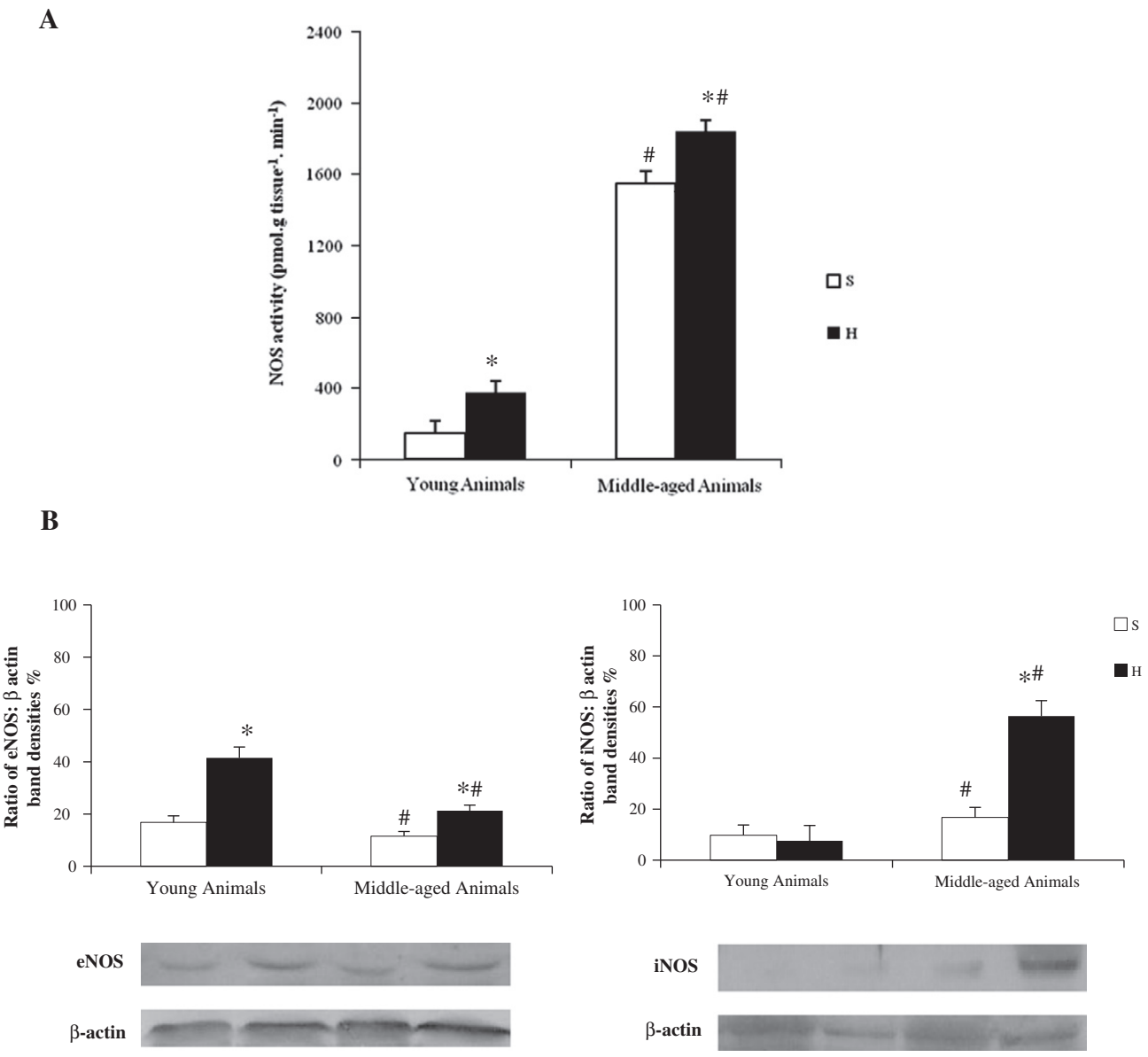

Fig. 2. A: NOS activity in ventricles of young and middle-aged rats subjected to acute hemorrhage. Values are mean ± SEM. *P<0.01 vs. the S group; #P<0.01 vs. the young animals from each group. B: Representative Western blots of eNOS and iNOS carried out on proteins from ventricles of young and middle-aged rats subjected to acute hemorrhage. Histograms illustrate mean eNOS and iNOS protein values for each group. Data are mean ± SEM. *P<0.05 vs. the S group; #P<0.05 vs. the young animals from each group. β-Actin served as an internal control.

determined using the Lowry assay. Equal amounts of protein samples (45 μg) were separated in 7.5% SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad, Munich, Germany), followed by a primary incubation with rabbit polyclonal anti-NOS antibodies (dilution 1:500: anti-iNOS, epitope at the carboxy terminus; anti-eNOS, epitope at the amino terminus; and anti-nNOS, epitope at the amino terminus; BD Biosciences, Franklin Lakes, NJ, USA) or with rabbit polyclonal anti-caveolin-1 antibody (dilution 1:500; Santa Cruz Biotechnology, San Diego, CA, USA), and a secondary immunoreaction with a goat anti-rabbit antibody conjugated with horseradish peroxidase (dilution 1:5000; Bio Rad, Munich, Germany). Samples were revealed by chemiluminescence for 2–4 min using an ECL reagent (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Band density was quantified by densitometric scanning of Western blots using a Hewlett–Packard scanner and Totallab analyzer software (Biodynamics Corp., Seattle, WA, USA), and protein amounts were calculated by comparison with the densitometric values of the corresponding β-actin bands. Protein levels were expressed as a ratio of the optical densities of the eNOS, iNOS and caveolin-1 bands and the β-actin band (BD Biosciences, Franklin Lakes, NJ, USA) to check for any inaccuracies in protein loading.

2.5. Immunofluorescence

Coimmunoprecipitation analysis was performed on the left ventricle at 60 min after bleeding (n = 4) to study the interaction between caveolin-1 and eNOS. Ventricles from groups S and H were fixed with a solution containing 4% paraformaldehyde in a phosphate-buffered saline (PBS) at 4 °C. The left ventricles were excised, sliced and postfixed overnight in the same fixative solution and then washed extensively. Fixed kidney blocks were infiltrated overnight in 30% sucrose and frozen at −80 °C. Cryostat sections (thickness: 16 μm) were obtained using a Leitz cryostat and collected on gelatine-subbed glass slides. After washing off PBS and fixation in acetone, slides were blocked with 10% normal goat serum in PBS for 60 min at room temperature. The slices were then incubated with mouse monoclonal anti-eNOS (dilution 1:500, BD Biosciences, Franklin Lakes, NJ, USA) and rabbit polyclonal anti-caveolin-1

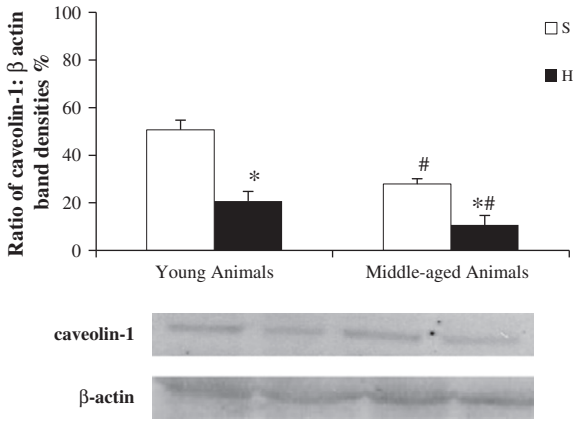

Fig. 3. Representative Western blots of caveolin-1 carried out on proteins from ventricles of young and middle-aged rats subjected to acute hemorrhage. Histograms illustrate the mean caveolin-1 protein values for each group. Data are mean ± SEM. *$P<0.05$ vs. the S group; #$P<0.05$ vs. the young animals from each group. β-Actin served as an internal control.

(dilution 1:500, Santa Cruz Biotechnology, San Diego, CA, USA) at 4 °C overnight. After washing off in PBS, the fluorescent secondary antibodies, rhodamine-labeled goat anti-rabbit IgG (dilution 1:50, Santa Cruz Biotechnology, San Diego, CA, USA) and fluoresceine-labeled goat anti-mouse IgG (dilution 1:50, Santa Cruz Biotechnology, San Diego, CA, USA), were applied and incubated for 1 h at room temperature. After washing off in PBS, the slides were mounted in a PBS/glycerol (3/1 vol/vol) solution before observation under a fluorescence microscope (Olympus BX 51). Control sections that were only incubated with the primary or secondary antibody showed no fluorescence (data not shown). Colocalization was visualized by superimposing the red caveolin-1 over the green eNOS using QCapture Pro 6.0 (QImaging Corporation, 2006), with the resultant yellow image representing the area of colocalization.

2.6. Statistical analysis

Data are mean ± SEM. The paired student's *T* test was used to compare changes in hemodynamic parameters. Analysis of variance of a variable (ANOVA) followed by a Bonferroni post-hoc test for multiple comparisons was used for the analysis of other data. The assumptions of normality, homogeneity of variances and independence of the data were checked. The 5% probability level was used as a criterion for significance. The software Prism (Graph Pad Software, San Diego, CA, USA) was used for statistical analysis.

3. Results

3.1. Changes in hemodynamic parameters during and after hemorrhagic state

Baseline MAP measurements (mm Hg) did not differ significantly among the four groups of animals (young rats: S = 84 ± 4; H = 92 ± 5; middle-aged rats: S = 87 ± 3, H = 93 ± 5; *P* = NS). The time course of MAP in young and middle-aged rats from group H is illustrated in Fig. 1A. Hemorrhage induced a marked decrease in MAP in the H group, and in young rats MAP dropped to 27 ± 3 mm Hg at 5 min following the bleeding period ($P<0.01$ vs. basal values), with subsequent stabilization at about 50 ± 5 mm Hg at 15 min ($P<0.01$ vs. basal values). Meanwhile, bleeding in middle-aged rats induced a decrease in MAP down to 24 ± 3 mm Hg at 5 min ($P<0.01$ vs. basal values, *P* = ns vs. young H rats) and this parameter reached a value of 45 ± 4 mm Hg at 15 min ($P<0.01$ vs. basal values, *P* = ns vs. young H rats). Hypotension was maintained until the end of the experimental period in both young and middle-aged rats. The baseline values of MAP and hypotension observed at 60 min after bleeding were similar in young and middle-aged rats, reflecting similar hemodynamic conditions. Fig. 1B illustrates the time course of HR in young and middle-aged H rats. Baseline HR measurements (bpm) did not differ significantly among the four groups of animals (young rats: S = 323 ± 7; H = 322 ± 6; middle-aged rats: S = 320 ± 7, H = 318 ± 8; *P* = NS). In young rats, after the reflex response, hemorrhage resulted in a brief decrease in HR in the first stage, followed by a gradual increase until 60 min (basal HR = 322 ± 6; HR 60 min = 352 ± 7*; *$P<0.01$ vs. basal values). Meanwhile, in middle-aged rats, bleeding induced a major decrease in HR followed by a gradual increase until 60 min (basal HR = 318 ± 8; HR 60 min = 377 ± 7*#; *$P<0.01$ vs. basal values; #$P<0.01$ vs. middle-aged H rats). Positive chronotropic effects observed after 60 min of bleeding were higher in adults than in young animals.

3.2. NOS activity

The potential ability to produce NO by the cardiac tissue was assessed by measuring NOS activity in the left ventricle of both young and middle-aged rats, as illustrated in Fig. 2A. In group S, ventricle NOS activity was significantly higher in middle-aged than in young animals. Hemorrhage increased NOS activity in young and middle-aged animals relative to S animals (61% and 16%, respectively).

3.3. Western blot of NOS isoforms

To assess the specificity of expressional regulation of NOS with hemorrhage and aging, variations in abundance of NOS isoforms in the left ventricle of all groups of animals were analyzed. In group S, eNOS protein levels were significantly lower (31%) in middle-aged rats than in young rats. However, iNOS abundance in middle-aged rats increased by about 70% compared with young ones. Hemorrhage enhanced eNOS protein levels at 60 min of bleeding in young and middle-aged animals compared with the S group (247% and 184%, respectively). Meanwhile, no changes in iNOS abundance between young animals in group H relative to group S were found. In middle-aged animals, bleeding increased iNOS abundance relative to group S (338%). No reaction with the anti-neuronal NOS antibody was observed in ventricles from S and H rats (Fig. 2B).

3.4. Changes in caveolin-1 abundance

As eNOS activity was shown to be regulated posttranslationally through its inhibitory interaction with the caveolar coat protein caveolin, potential changes in the abundance of caveolin-1 expressed in endothelial cells and cardiomyocytes were assessed by Western blot analysis. In group S, ventricular caveolin-1 protein levels were significantly lower (33%) in middle-aged rats than in young rats. Hemorrhage decreased caveolin-1 abundance 60 min after bleeding in young and middle-aged animals relative to group S (58% and 63%, respectively) (Fig. 3).

3.5. Immunoprecipitation assay for e-NOS and caveolin-1 interaction

eNOS was immunoprecipitated with anti-caveolin-1 antibodies from whole ventricle extracts of young and middle-aged rats to assess eNOS potential for association with the inhibitory protein caveolin-1 in rat ventricles in situ. Caveolin-1 and eNOS are present mainly in endothelial and smooth muscle cells. Colocalization studies showed that there is a diffuse pattern in young S rats. This localization pattern is associated with caveolin-1. A marked dissociation between caveolin-1 and eNOS was observed at 60 min after acute hemorrhage of 20% of the total blood volume (Fig. 4A). The localization pattern of eNOS and caveolin-1 in ventricles of middle-aged S animals showed greater dissociation than in young animals of the same group. This feature was also observed after 60 min of bleeding (Fig. 4B).

4. Discussion

The present study demonstrates the involvement of the allosteric modulator caveolin-1 in the regulation of cardiac NOS activity in hypovolemic state and with aging.

The major new finding is that myocardia from young and middle-aged rats subjected to hemorrhage-induced hypovolemia exhibited an increase in NOS activity and protein levels, as well as a decrease in abundance of caveolin-1 accompanied by greater dissociation between eNOS and its regulatory protein. It is known that the aging process is associated with several physiological changes in cardiomyocytes that result in loss of contractile function and the loss of endogenous protection against sickness or injury [22]. In our study, the baseline values of MAP and HR were similar for all young and middle-aged animals at the beginning of the experiments, reflecting similar hemodynamic conditions. Within 5 min after bleeding, hemorrhage (20% of the volemia) elicited a significant decrease (65%) in arterial blood pressure compared with basal values in both young and middle-aged rats in the H group. After this, this parameter stabilized at 49 ± 5 mm Hg 60 min after blood withdrawal. These findings suggest that hemorrhage-induced hypovolemia would be age independent. With regard to HR, we have shown that, after the expected immediate reflex-induced tachycardia, hypovolemic state induced a bradycardic stage followed by a gradual rise in HR in both H groups at 60 min after bleeding (young 9%; middle-aged 19%). It is known that blood pressure is maintained in the early stage of hemorrhage by a reflex increase in HR, vascular resistance, and peripheral sympathetic nerve activity. With aging, both baroreflex sensitivity [23] and vagally mediated cardiac variability [24] become attenuated, and sympathetically mediated fluctuations in vascular tone become prominent. Nevertheless, we did not observe significant differences in reflex-induced tachycardia either in young or in middle-aged animals. The ensuing bradycardia may result from a reflex complex that may develop in order to reduce an ongoing blood loss by reducing blood pressure through peripheral vasodilatation and, at the same time, to maintain

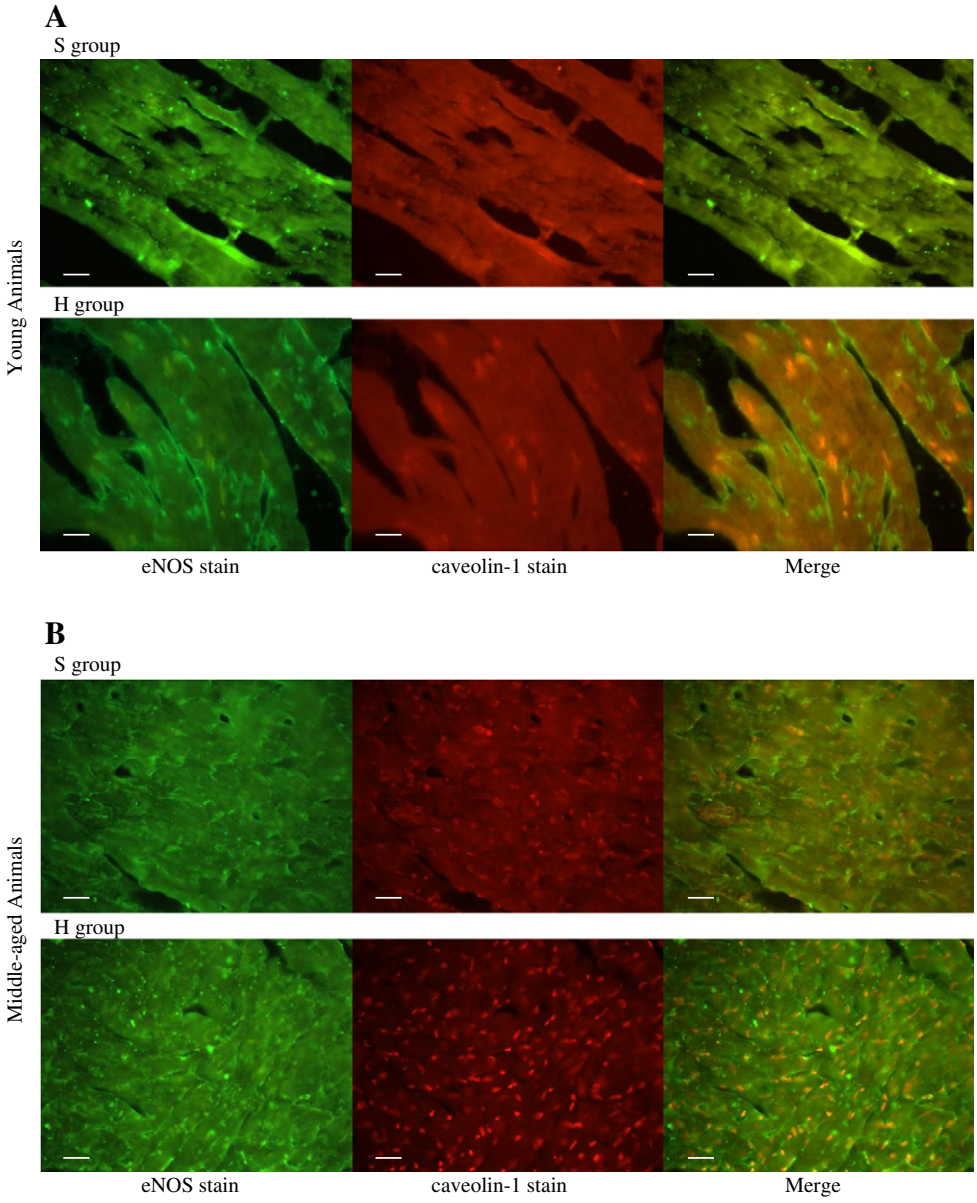

Fig. 4. Caveolin-1 and eNOS distribution in ventricles of young and middle-aged rats subjected to acute hemorrhage. Rat ventricle sections were double immunolabeled with anti-eNOS monoclonal antibody and anti-caveolin-1 polyclonal antibody. Colocalization of eNOS and caveolin-1 is shown in merged images. A. Young animals: note that a diffuse eNOS localization pattern in ventricular cells is associated with caveolin-1. After bleeding, dissociation between caveolin-1 and eNOS was observed. B. Middle-aged animals: the ventricles showed a localization pattern of eNOS and caveolin-1 with greater dissociation than in young animals of both the S and H groups (bar = 20 μm).

organ blood flow. There is evidence indicating that aging could influence the levels of NOS and the production of NO [25]. In consonance with other studies performed in adult rats [26,27], we found that NOS activity in the left ventricle is remarkably upregulated in middle-aged animals compared with young ones. In light of our findings, it is plausible that advancing age in itself may alter the NO system in order to maintain normal ventricle homeostasis. Additionally, Western blot analysis revealed that the protein expression of eNOS was lower in middle-aged S rats compared with young S animals. It should also be mentioned that increased NOS activity was also observed to be associated with elevated iNOS protein levels in middle-aged rats compared with young ones. Given that iNOS expression is frequently accompanied by an increase in reactive oxygen species and cytokines [28], being oxidative stress as an important factor contributing to the aging process, enhanced formation of cardiac iNOS-derived NO could account for aged heart function. Hierholzer et al. [29] have demonstrated that iNOS expression is upregulated during hemorrhagic shock and that the resulting increased production of NO produces a range of deleterious responses and end-organ damage. Previous data from our laboratory indicate that iNOS becomes a major source of cardiac NO production only in the later stages of hemorrhagic shock in young animals [4]. Indeed, the present results show an increase in iNOS protein levels after 60 min of bleeding only in middle-aged rats. We posit that age would be a major factor in the activation of the iNOS/NO pathway in early ventricular adjustment to hypovolemic state after hemorrhage. Several studies demonstrate that the effect of higher amounts of NO on cardiac inotropic state is negative, for example, in sepsis [30] and in mice overexpressing large amounts of eNOS [31]. The lower increase in ventricular NO after hemorrhage observed in middle-aged animals could be responsible for the larger positive chronotropic effect. However, we cannot rule out the integrated mechanisms which become activated in response to hemorrhage or the release of several neurohormonal vasoconstrictor factors (catecholamines, endothelins, vasopressin, renin–angiotensin system) [32,33].

Furthermore, given that eNOS is the primary physiological source of NO in the cardiovascular system and it is negatively modulated by caveolin-1, we examined expressional changes in caveolin-1 abundance as well as the functional consequence of aging. Ostrom and Insel [34] suggest that changes in caveolin expression could alter cell function with aging, and Kawabe et al. [35] have shown variable changes in caveolin-1, -2 and -3 expression with aging and maturation in heart, lung and skeletal muscle. The present results show lower amounts of caveolin-1 in middle-aged than in young rats. In addition, we observed an increase in dissociation of caveolin-1 from ventricle eNOS with age. The downregulation of caveolin-1 levels and partially disassembling from eNOS observed could constitute one of the mechanisms leading to over-activated NOS during the aging process. It is important to note that NO generated by eNOS plays a vital physiological role in maintaining appropriate microvascular tone and blood flow, and exhibits protective effects [36]. Confirming our previous studies conducted in young rats [4], we found that NOS activity was upregulated by the largest amount of eNOS after 60 min of bleeding, accompanied by a decrease in a negative regulatory protein, caveolin-1, in both young and middle-aged animals. Certain pathways for downregulating caveolin-1 expression (MAP kinase pathways, Src family kinases) and post-translational modifications such as ubiquitination and phosphorylation have emerged as important regulators of protein stability and function [37–39]. These mechanisms would explain, at least in part, how caveolin-1 protein levels are downregulated in hemorrhaged rats. A decrease in or absence of caveolin-1 was shown to potentiate basal and agonist-stimulated eNOS activities [40,41]. Aside from absolute changes in caveolin abundance, it is also important to consider whether the alteration in the tissue and subcellular distribution of caveolin-1 would influence NOS activity. In the present study we have shown that the decrease in total caveolin-1 abundance is also paralleled by decreased interaction with eNOS, measured by eNOS coimmunoprecipitated with caveolin-1 in hemorrhaged-ventricles from young and middle-aged animals. For maximal activation of NO release to occur locally in response to shear stress, growth factors or calcium-mobilizing agonists, it is possible that caveolin dissociates from eNOS or that other regulatory proteins are recruited to the complex to relieve the inhibition imposed by caveolin binding [18]. Studies elucidating the interactions between caveolin-1 and eNOS after stimulation of NO release and the dynamic trafficking of eNOS to and from the caveolae will reveal more functional roles for this important interaction. Consequently, these results emphasize the need to integrate the changes in protein cellular compartmentalization into the molecular program characterizing hypovolemic state and aging.

In conclusion, the present data provide evidence that aging and acute hemorrhage contribute to the development of upregulation of ventricular NOS activity. We have demonstrated that specific expression patterns of NOS isoforms, alterations in the amount of caveolin-1 and caveolin-1/eNOS interaction are involved in aged-related adjustment to hypovolemic state.

Acknowledgments

This study was supported by Universidad de Buenos Aires grant B022. The authors wish to thank Sandra Landin for secretarial work, the School of Pharmacy and Biochemistry, Universidad de Buenos Aires, and English teacher Ana Borthwick.

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