Caveolin-1 and -3 dissociations from caveolae to cytosol in the heart during aging and after myocardial infarction in rat

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Abstract

Objective: Caveolins, the structural proteins of caveolae, modulate numerous signaling pathways including Nitric Oxide (NO) production. Among the caveolin family, caveolin-1 and -3 are mainly expressed in endothelial and muscle cells, respectively. In this study, we investigate whether (i) changes in caveolin abundance and/or distribution occur during cardiac aging and failure in rat, and (ii) the process could influence NO synthase (NOS) activity.

Methods: Using immunohistolabelling and Western blot approaches, expression and distribution of caveolins were analysed in adult (Ad), senescent (S-Sh) and myocardial infarction-induced failing (S-MI) hearts. NOS3/caveolin-1 interactions were evaluated by immunoprecipitation assays.

Results: At the microscope level, caveolin-1 distribution in the endothelial cells was unchanged between the groups. Conversely, the typical distribution of caveolin-3 in myocyte sarcolemma was dramatically altered in S-MI rats, resulting in a heterogeneous pattern throughout the septum. Total abundance of caveolin-1 and -3 remained stable whatever the group. In the fractions free of caveola (Triton X-100 soluble), the levels of caveolin-1 and -3 increased with aging (+20%, and +104%, P < 0.05 versus Ad, respectively) and were further enhanced in S-MI (+25%, +30%, P < 0.05, P < 0.001 versus S-Sh respectively). In these fractions, NOS3/caveolin-1 complexes increased as well. In addition, NOS activity was negatively correlated to caveolin-1 level in the cytosolic fractions.

Conclusions: We demonstrate that dissociation of caveolin from caveolae is associated with aging and heart failure, the process being related to the decreased NOS activity.

Keywords: Aging; Capillaries; Heart failure; Sarcolemma

1. Introduction

The cardiac remodelling associated with heart failure (HF) includes important modifications of both cellular and subcellular organisation [1]. The importance of membrane microdomains such as caveolae in signal transduction is demonstrated in vessels and myocardium [2–4]. The caveola coat protein caveolin exists as different isoforms, the expression of which is tissue specific. In the heart, caveolin-1 and -3 are mainly expressed in endothelial cells and cardiomyocytes, respectively, as reviewed in [2,5]. Within caveolae, caveolin-1 and -3 are involved in signal transduction by ensuring the compartmentalisation of signalling molecules such as G-proteins and tyrosine kinase-associated receptors as well as Nitric Oxide synthase (NOS), reviews in [2,5,6]. Although caveolin-1 and -3 emerge as key elements implicated in signal transduction, only a few studies have specifically examined expression changes in caveolin abundance as well as the functional consequences in diseased heart. Abundance of caveolin-3 but not caveolin-1 is increased in failing dog hearts [7].
Decreased expression of caveolin-3 and -1 is observed in a model of hypertrophic cardiomyopathy in dogs [8] whereas in the spontaneously hypertensive rat, only the downregulation of caveolin-3 is demonstrated [9]. In view of these apparent discrepancies, the question arises whether in the heart caveolin expression is differentially regulated according to the patho-physiological situation. This issue is of potential importance since absolute changes in caveolin-3 and -1 abundance are not only expected to influence cardiomyopathy development [10] but also NOS3 activity [11], and thus the development of endothelial dysfunction, a major event involved in the pathogenesis of heart failure [12]. A decrease in or an absence of caveolin-1 was shown to potentiate basal and agonist-stimulated NOS3 activity [13–15]. Such a relation between caveolin-1 abundance and NOS activity was not found in a model of cardiac hypertrophy in dogs [8]. Aside from absolute changes in caveolin abundance, whether alterations in the tissular and subcellular distribution of caveolin influence NOS activity have not been investigated in in vivo models of heart disease.

In light of the fact that both heart failure and endothelial dysfunction are more frequent during aging, we have analysed both the expression and the distribution of caveolin isoforms in adult and senescent rat heart as well as in an experimental model of myocardial infarction-induced heart failure in the aged rat. We took advantage of the Triton X-100 insolubility of caveolae [16] to analyse the subcellular distribution of caveolins in the heart. In addition, we investigated whether alterations in the caveolin distribution and caveolin/NOS3 interaction were related to NOS dysfunction during aging and chronic heart failure.

2. Experimental procedures

2.1. Materials

The following materials were purchased from the indicated sources: nitrocellulose transfer membrane (0.45 μm pore), Schleicher&Schuell; antibodies: mouse anti-caveolin-1 (clone 2297), mouse anti-caveolin-3 (clone 26) and mouse anti-NOS3 (clone 33), Transduction Laboratories; rabbit anti-caveolin-1α (sc-894), and rabbit anti-NOS3 (sc-654), Santa Cruz; rabbit anti-laminin, Chemicon; fluorescein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulin G, Texas-red-conjugated donkey anti-rabbit polyclonal IgG, anti-mouse IgG- and anti-rabbit IgG-conjugated to horseradish peroxidase, Amersham Pharmacon. Vectashield mounting medium containing 4,6-diamidino-2-phenylindole (DAPI), Vector. Anti-mouse IgG-conjugated Agarose, Sigma. Protein A-conjugated Agarose, Transduction Laboratories. Enhanced chemiluminescence (ECL+) immunoblotting reagents, Amersham. BCA protein assay kit, Pierce. [3H]-l-arginine, NEN/DuPont; Tetra Hydro-bioterin; Research Biomedical. Ethylenediaminetetra-acetic acid (EDTA), Ethylene Glycol-bis(Baminoethyl Ether)N,N,N',N'-Tetraacetic acid (EGTA), Sigma. Ethylenophenylpolyethylene glycol (NP40), Fluka.

2.2. Animals

The investigation was performed in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). Wistar rats (Charles River France) were used (7–8 animals per group) in the study. Myocardial infarction (S-MI), obtained by coronary artery ligation, and sham operation (S-Sh) were performed on 24 month-old rats as described in [17]. S-Sh and S-MI rats were analysed 3 months after surgery. In parallel, 3- (adult, Ad) and 27- (senescent, S) month-old normal rats were included in the protocol. Ad served as a control group of aging. S-Sh served as a control group of S-MI.

2.3. Hemodynamic study

Animals (Ad, S-Sh and S-MI) were anesthetised and hemodynamic parameters were measured as described in [17]. Briefly a catheter pressure transducer (model TC50, Millar instrument) was connected to the data acquisition unit (MP100, Biopac systems). We studied first derivative of left ventricular pressure (+dP/dt max), left ventricular end diastolic pressure (LVEDP) and others cardiac parameters. The hearts were removed and weighed. The left ventricle (LV) was sectioned transversely into three slices from the apex to base and rapidly frozen in isopentane precooled in liquid nitrogen, then stored at −80°C.

2.4. Immunohistochemistry

Cryostat sections of heart tissue (7 μm thick) were postfixed in acetone/methanol (1:1) at −20°C. To prevent nonspecific binding after rinsing in phosphate-buffered saline (PBS, pH 7.4), sections were preincubated in PBS containing 5% bovine serum albumine (BSA) for 30 min at room temperature (RT). Monoclonal antibodies (mAb) to caveolin-1 (1:50), caveolin-3 (1:100) or polyclonal antibodies (pAb) to caveolin-1α were applied to sections for 1 h at RT. After three washes in PBS, slides treated with mAb were incubated with anti-laminin pAb (1:100), 1 h at RT. Binding of primary antibodies was detected by incubating the sections, 1 h at RT, with FITC-conjugated anti-mouse IgG (1:40) and Texas-Red-conjugated anti-rabbit IgG (1:40). Caveolin-1α antibodies were detected by incubating the sections, 1 h at RT, with FITC-conjugated anti-rabbit IgG (1:40). Sections were finally washed as above and mounted in Vectashield medium. Fluorescence was observed using either a DMRB Leica microscope equipped with epifluorescence optics or a Nikon E600FN upright fluorescence microscope equipped with a
Bio-Rad MRC-1024/2-P multiphoton imaging attachment in confocal mode.

2.5. Morphometric analysis

Morphometric image analysis was applied to assess quantitatively the structural changes of the hypertrophied LV septum wall using a computer-assisted image-analysis system device (Optilab, Graftek, Germany). Laminin labeled sections were used to assess cardiomyocyte width. Fields were accepted for quantitative analysis if cardiomyocytes (a) were seen in cross sections, (b) had a visible nucleus, and (c) had intact cellular membranes. Cardiomyocyte width was assessed accordingly by marking the border of the cells using the image-analysis system.

2.6. Protein extractions

Three LV cryostat sections (15 μm width) free of infarction scar were lysed in boiling sodium dodecyl sulfate (SDS) buffer (1% SDS, in mmol/l: 10 Tris–HCl pH 7.4, 1 ortho-vanadate, 0.1 leupeptine, 0.001 aprotinine, 10 mg/ml PMSF). Lysates were boiled in a microwave oven for 15 s (900 W) and clarified by centrifugation at 11,000 g for 15 min at 15 °C. In parallel, the same amount of tissue was homogenized in Triton X-100 buffer (1% Triton X-100, in mmol/l: 50 Tris–HCl pH 7.4, 100 NaCl, 50 NaF, 5 EDTA, 40 β-glycerophosphate, 0.2 ortho-vanadate, 0.1 leupeptine, 0.001 aprotinine) for 1 h at 4 °C. After centrifugation (22,000 g, 15 min) at 4 °C, supernatants were collected (Triton X-100 soluble fraction). Because of its property to obtain undenatured total caveolin pools [18], OG lysis buffer was employed to analyse protein–protein interactions. Three LV cryostat sections were lysed in OG buffer (in mmol/l: 600 N-0-cetylglucosamide, 10 Tris–HCl pH 8, 150 NaCl, 10 NaF, 1 ortho-vanadate, 0.1 leupeptine, 0.001 aprotinine) for 1 h at 4 °C. After centrifugation (15,000 g, 5 min) supernatants were collected. Lysate protein concentrations were measured using the BCA protein assay kit, and the samples were stored at −20 °C until use.

2.7. Western blot analysis

The cellular contents in caveolin-1, -1α and -3 were determined by Western blot analysis. Proteins (20 μg) to detect caveolin-1 and -3; 5 μg to detect caveolin-1α) were resolved by electrophoresis on 12% SDS-acrylamide gel and transferred to nitrocellulose membrane in 25 mM Tris, 192 mM glycine, 0.01% SDS, 15% methanol. The membranes were blocked with Tris-buffered saline (TBS, pH 7.4) containing 0.1% Tween-20, 5% non-fat dry milk and 1% BSA, prior to incubation, 1 h at RT, with either mAb anti-caveolin-1 (1:5,000), mAb caveolin-3 (1:10,000), or pAb caveolin-1α (1:10,000) in TBS-Tween-20. After washing, membranes were incubated (1 h at RT) with either anti-mouse IgG- or anti-rabbit IgG-conjugated to horseradish peroxidase (1:5,000). After washing, immunoreactive bands were visualized by enhanced chemiluminescence ECL+ and quantified by densitometry using a computer-based imaging system (Gel Doc 1000; Biorad).

2.8. Measurement of NOS activity

Total NOS activity was measured by the conversion of (14)H-n-arginine to (14)H-n-citrulline, according to [19]. Rat hearts were homogenized in a buffer described in [20]. Initial experiments showed that the reaction was linear for 20 min, and was concentration-dependently inhibited by addition of the NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME).

2.9. Co-immunoprecipitation

For immunoprecipitation (IP), rat heart lysate proteins (300 μg) were incubated overnight at 4 °C with either mAb caveolin-1 or mAb NOS3 at a final concentration of 5 μg/ml in 400 μl of IP buffer (1% Triton X-100, 0.5% Nonidet NP-40, and in mmol/l: 150 NaCl, 10 Tris–HCl pH 7.4, 1 EDTA, 0.2 ortho-vanadate, 0.2 PMSF). Anti-mouse IgG-conjugated Agarose (50 μl) or Protein A-conjugated agarose (15 μl) were added for 3 h at 4 °C. After three washes with IP buffer, the immunoprecipitates were eluted in Laemmli buffer and separated in two parts for Western blot analysis. Samples were separated by electrophoresis and immunoblotted with pAb caveolin-1α (1:10,000) or pAb NOS3 (1:2500)

2.10. Statistical analysis

ANOVA tests were used in statistical evaluation of the data, which are given as mean±S.E.M. A Scheffe-test, except when indicated, was used, and a value of P < 0.05 was considered as significant.

3. Results

3.1. Animal characteristics

Body weights, heart weights and baseline hemodynamic parameters of animal groups are shown in Table 1. With aging, both heart weight and myocyte width significantly increased compared with adults whilst contractile properties (+dP/dt) slightly decreased in the S-Sh group indicating moderate alterations of myocardial function associated with aging. In the S-MI group, the heart weight to body weight ratio was higher than that of age-matched sham-operated rats. LVEDP was markedly increased in S-MI. These morphological and physiological parameters, which are in close agreement with those described in [21],
Table 1
Hemodynamic and anatomical parameters

<table>
<thead>
<tr>
<th></th>
<th>Ad (n = 7)</th>
<th>S-Sh (n = 7)</th>
<th>S-MI (n = 8)</th>
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<tbody>
<tr>
<td>BW (g)</td>
<td>380±5</td>
<td>678±15 *</td>
<td>578±30 **</td>
</tr>
<tr>
<td>HW (mg)</td>
<td>859±18</td>
<td>1357±69 *</td>
<td>1414±144 *</td>
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<td>HW/BW</td>
<td>2.21±0.02</td>
<td>1.97±0.03</td>
<td>2.62±0.25 †</td>
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<tr>
<td>LVEDP(mmHg)</td>
<td>8.73±0.75</td>
<td>12.28±1.64 *</td>
<td>16.92±1.12 ‡</td>
</tr>
<tr>
<td>+dp/dt (mmHg)</td>
<td>5483±235</td>
<td>3889±274 §</td>
<td>3924±417 $</td>
</tr>
<tr>
<td>Myocyte Ø (µm)</td>
<td>16.7±0.8</td>
<td>21.1±0.4 §</td>
<td>23.5±1.8 $</td>
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* P < 0.01 versus S-Sh; ** P < 0.001 versus S-Sh; † P < 0.05 versus Ad; ‡ P < 0.05 versus Ad; § P < 0.001 versus S-Sh.

Table 1 munolabelling with caveolin-3 and laminin antibodies clearly indicates that caveolin-3 was present at sarcolemma and intercalated disk levels of all cardiomyocytes (Fig. 3A and B). In senescent heart, caveolin-3 distribution was similar to that found in Ad ventricular myocardium (Fig. 3C). After myocardial infarction, caveolin-3 labelling disappeared from some myocyte sarcolemma (arrows) whereas others remained labelled throughout the septum (Fig. 3E). Laminin staining indicated that myocytes with caveolin-3 disappearance were not degenerating cells (Fig. 3F). The defect in caveolin-3 distribution was further assessed by confocal microscopy analysis (Fig. 4). In the adult heart, the pattern of distribution was identical to that already described (Figs. 3A and 4A), however a slight discontinuous labelling of sarcolemma could be detected in senescent rat heart sections (Fig. 4B). In S-MI heart, caveolin-3 distribution was also altered, resulting in heterogeneous pattern throughout the section (Fig. 4C).

Notably, in S-MI cardiomyocytes free of caveolin-3 at the sarcolemmal level, no intracellular organelles were labelled with caveolin-3Ab.

3.2. Caveolin distribution in Ad, S-Sh and S-MI rat hearts

To define antibody specificities, protein lysates from endothelial cells and cardiomyocytes were analysed using Western blot (Fig. 1). For caveolin-1 mAb, caveolin-1-a pAb, and caveolin-3 mAb a signal was only detected in endothelial cells and cardiomyocytes, respectively.

Using conventional fluorescent immunolabelling, caveolin distribution was analyzed in the different groups. In accordance with the literature [3,22], caveolin-1 was detected in endothelial cells and to a lesser extent in smooth muscle cells of the coronary artery media in Ad rat (Fig. 2A). No significant change in either distribution or staining intensity was observed in the two senescent animal groups (Fig. 2B and C). Caveolin-1a was expressed in endothelial cells, its distribution being identical to that of caveolin-1 in all rat groups (Fig. 2D–F). In longitudinal cryosections of adult rat heart, double immunolabelling with caveolin-3 and laminin antibodies clearly indicates that caveolin-3 was present at sarcolemma and intercalated disk levels of all cardiomyocytes (Fig. 3A and B). In senescent heart, caveolin-3 distribution was similar to that found in Ad ventricular myocardium (Fig. 3C). After myocardial infarction, caveolin-3 labelling disappeared from some myocyte sarcolemma (arrows) whereas others remained labelled throughout the septum (Fig. 3E). Laminin staining indicated that myocytes with caveolin-3 disappearance were not degenerating cells (Fig. 3F). The defect in caveolin-3 distribution was further assessed by confocal microscopy analysis (Fig. 4). In the adult heart, the pattern of distribution was identical to that already described (Figs. 3A and 4A), however a slight discontinuous labelling of sarcolemma could be detected in senescent rat heart sections (Fig. 4B). In S-MI heart, caveolin-3 distribution was also altered, resulting in heterogeneous pattern throughout the section (Fig. 4C).

Notably, in S-MI cardiomyocytes free of caveolin-3 at the sarcolemmal level, no intracellular organelles were labelled with caveolin-3Ab.

3.3. Caveolin-3 dissociation from caveolae during aging and heart failure

For further insights into the changes in subcellular distribution and expression of caveolin-3 under the different patho-physiological conditions, Western blots were performed (Fig. 5). The relative level of caveolin-3 expression was evaluated in SDS lysates, which represent the total caveolin-3 pool (Fig. 5A and B). In parallel, taking advantage that caveolins associated to caveolae are Triton X100 soluble at 4°C whereas cytosolic and Golgi apparatus associated-caveolins are Triton X-100 soluble [16,23], we analysed the amount of caveolin-3 in the Triton X-100 soluble fractions (Fig. 5C and D). In SDS lysate, caveolin-3 in Ad heart tissues was present in significant amounts and its level remained relatively constant in the three animal groups, the small decrease in the S-MI group being not statistically significant (P = 0.16 versus S-Sh) (Fig. 5B). In contrast, the amount of caveolin-3 in the Triton X100-soluble fraction, that represented less than 25% of total caveolin, increased (+20%, P < 0.05) in the S-Sh when compared to the Ad group (Fig. 5D). To assess that surgery was not involved in the process, the level of caveolin-3 was analysed in the Triton X100-soluble cardiac fraction of 27 month-old animals (n = 6), and its amount (1.10±0.10 A.U.) was similar to that of S-Sh group (1.06±0.11 A.U.). The value further augmented in the S-MI group (+25%, P < 0.05, versus S-Sh). On the other hand the caveolin-3 amount in the Triton insoluble fractions did not vary among groups (Fig. 6).

The data clearly indicate that caveolin-3 abundance in the rat ventricle remained similar in the different patho-physiological states but that a part of the protein disassem-
Fig. 2. Caveolin-1 and -1α distributions in adult (A and D), senescent (B and E) and infarcted-senescent (C and F) rat hearts. LV sections were labelled with anti-caveolin-1 mAb (A, B and C) or anti-caveolin-1α pAb (D, E and F). Note that caveolin-1, like caveolin-1α is expressed only in endothelial cells. (A–C: bar = 20 μm and D–F: bar = 40 μm).

bles from the caveolar structure with aging, this process being amplified in the failing heart.

3.4. Dissociation of caveolin-1 and -1α from caveolae during aging and heart failure

A similar quantitative approach was used to analyse the expression and the subcellular distributions of both caveolin-1 and -1α in the experimental groups. In SDS-lysate, no significant changes in caveolin-1 and -1α amounts were observed in senescent heart and after myocardial infarction (P = 0.858) (Fig. 7A and B). In the Triton X-100 soluble fraction (Fig. 7C and E), both caveolin-1 and -1α were weakly detectable in Ad group. The amount of caveolin-1 increased (+176%, P = 0.011 versus S-Sh) in the S-MI group only whereas that of caveolin-1α significantly increased (+104%, P = 0.014 versus Ad) with aging and was further enhanced (+30%, P < 0.001 versus S-Sh) after myocardial infarction (Fig. 7D and F). In the Triton X-100 insoluble fraction, caveolin-1 and caveolin-1α amounts did not vary among animal groups (Fig. 7).

These data demonstrate that the caveolin-1 abundance in the heart did not vary between groups but that, for a
significant proportion of the isoform-1α, changes in the subcellular distribution occurred during aging, the process being amplified in the failing heart.

**3.5. Relationship between NOS and caveolin-1**

To investigate the functional consequences of caveolin-1...
Fig. 4. Decreased expression of caveolin-3 at sarcolemma level of myocytes in senescent and infarcted-senescent transversal rat heart sections. Heart sections from adult (A), senescent (B) and infarcted-senescent rats (C) were labelled with anti-caveolin-3 mAb. Confocal fluorescent microscopy confirmed the caveolin-3 disappearance from some myocytes in infarcted myocardium (C) when compared to normal heart (A,B). Note the transverse staining (heads of arrow, panel A) which revealed a T-tubule label in adult heart. (Bar = 20 μm).

Fig. 5. Caveolin-3 abundance in total lysates (A and B) and Triton X-100 soluble materials (C and D) of adult, senescent and infarcted-senescent rat hearts. (A) SDS lysates were loaded onto gel and membranes were incubated with anti-caveolin-3 mAb. (B) is a Bar graph summarizing caveolin-3 protein levels in SDS lysates. (C) and (D) showed respectively a representative Western blot of the Triton X-100-soluble material using anti-caveolin-3 mAb and the corresponding quantification. S-Ponceau staining of each membrane confirmed that equal amounts of protein were loaded. Ad, adult; S-Sh, senescent-sham; S-MI, senescent-myocardial infarction. * P < 0.05, n = 6 per group.
3.6. Relationship between cardiac growth and caveolin dissociation from caveolae

To begin to unravel mechanisms involved in the caveolin redistribution process during patho-physiological situations, we investigated potential correlations between physiological parameters and amounts of caveolin in different experimental conditions. Positive linear correlations were found between the amounts of either caveolin-1 or caveolin-1α in the Triton X-100-soluble fraction and heart weight (Fig. 9A and B; n = 14; r = 0.551, P = 0.04 and r = 0.818, P = 0.0004, respectively). Similarly, the amount of caveolin-3 in the Triton X-100 soluble fraction correlated to both heart weight and myocyte diameter (Fig. 9C and D; n = 13, r = 0.729, P = 0.0047 and n = 12, r = 0.670, P = 0.017, respectively). As expected, no relation between heart weight and the amounts of either caveolin-1 or caveolin-3 in SDS lysates was found (not shown). Notably, neither caveolin-3 nor caveolin-1 levels in the Triton-soluble fraction were related to parameters of myocardial dysfunction such as +dP/dt or LVEDP.

4. Discussion

The present study demonstrates that caveolin-1 and caveolin-3 dissociate from caveolae within cardiac cells in accordance with patho-physiological conditions. Three lines of experimental evidence support these conclusions: first, the disappearance of caveolin-3 from sarcolemma in failing heart demonstrated by immuno-localization; second, the increasing amounts of both caveolin-1 and -3 into the Triton-X-100 soluble material without any variation in their respective basal expression; third, the increased amount of caveolin-1/NOS3 complexes in Triton-X-100 soluble fractions. The alterations are specifically associated with either aging or aging-associated heart failure and have functional consequences as evidenced by the negative correlation existing between the amount of caveolin-1α in the cytosolic fraction and NOS activity.

The changes in hemodynamic parameters indicated that aged myocardium is a moderately diseased heart as previously proposed [1] whereas after myocardial infarction, animals exhibited a significant increased LVEDP but no major decrease in inotropic properties indicating a moderate stage of cardiac failure [21]. Therefore, these experimental models allowed us to discriminate alterations in caveolin distribution associated with senescence from those related to cardiac failure.

In adult cardiac tissue, both immunological and biochemical data relative to caveolin-1 and -3 expression and distribution are in close agreement with previous studies [3,22]. Under normal conditions, the proportion of caveolin in the Triton X-100 soluble material is minute...
whatever the isoforms considered, as already described [24]. Caveolin-1 and its α-isofom are mainly detected in endothelial and smooth muscle cells. The superimposable caveolin-1 and caveolin-1α patterns of distribution indicate that the two isoforms are similarly distributed within cardiac tissue. Caveolin-3 is detected in the intercalated disk and sarcolemma, as previously described [22].

A major finding of this study is that aging and cardiac failure are associated with caveolin-3 dissociation from sarcolemma caveolae. First, using either conventional or confocal fluorescent microscopic approaches (Figs. 3 and 4), we demonstrate a disappearance of caveolin-3 from the...
buted into cytosol in aging and cardiac failure. This result, not only signifies its dissociation from caveolae, but could also indicate an increase of caveolin monomer in the cytosol fraction although an association to either Golgi apparatus and/or integrin cannot be excluded [24,25]. However, no relocation of caveolin-3 within myocyte subcellular structure such as Golgi apparatus could be detected by immunofluorescence. This led us to propose that a part of caveolin-3 dissociates from caveolae into cytosol, the process being initiated with aging and amplified by myocardial infarction-induced hypertrophy. In addition, we demonstrate that no major changes in caveolin-3 expression occurred in ventricles during either aging or heart failure. The present results differ from those of Hare et al. [7] who have reported an increased expression of caveolin-3 in dog failing heart. The discrepancy might be due to the etiology of cardiac failure since chronic pacing results in a dilated cardiomyopathy whereas myocardial infarction is associated with hypertrophy of surviving tissue before the appearance of cardiac failure signs [26]. Since tight regulations in the amount of caveolin-3 [27,28], and its association with molecular partners [29] are essential for maintaining normal muscle homeostasis, the caveolin-3 dissociation from caveolae shown herein might have dramatic consequences for cardiomyocyte signalling such as via the α/β-adrenergic pathways [9,30].

The other major finding of the present study is that cardiac failure is associated with alterations in caveolin-1 subcellular distribution. Two caveolin-1 isoforms, α and β, have been described [31]. However, immunological approaches are unable to specifically detect the β-isoform since the two caveolin-1 isoforms only differ by an additional N-terminal sequence of 31 amino-acids in the α-isoform [31]. As observed in dilated cardiomyopathy [7], the total amount of caveolin-1, as well as the respective proportion of caveolin-1α and -1β, does not vary with either aging or cardiac failure (Fig. 7). However the amount of caveolin-1α and particularly that of the isoform-1α increased in the Triton X-100 soluble fractions of failing senescent heart. The increased level of caveolin-1α but not caveolin-1 detected in senescent rat heart soluble fraction, suggests that the process of caveolin-1 dissociation from caveolae affects mostly the α-isoform, the process being related to the development of cardiac hypertrophy (Figs. 7 and 9). Due to the narrowness of endothelial cells, immunofluorescence was unsuccessful in detecting caveolin-1α disappearance from endothelial cell membrane as observed in myocytes for caveolin-3.

Given the fact that caveolin-1 ensures the efficient activation of NOS3 upon agonist stimulation [5], the dissociation of caveolin-1α from caveolae that we demonstrated herein has the potential to alter NOS3 activity. It has been shown that caveolin-1, when present within caveolae binds to NOS3 and inhibits enzyme’s activity in a
process that could be reversed by Ca\textsuperscript{2+}-activated calmodulin [32]. Recently Feron et al. [13] showed that a decrease in caveolin-1 potentiated basal and agonist-stimulated NOS3 activity. Investigating whether a relation existed between the caveolin-1\textsubscript{α} dissociation from caveolae and NO production we showed that (i) caveolin-1\textsubscript{α} levels in the Triton X-100 soluble fraction was inversely related to NOS activity (Fig. 8A), and (ii) the amount of caveolin-1/NOS3 complexes increased in the cytosol of failing heart whereas the total pool of complexes did not vary (Fig. 8B). We might postulate that when present in cytosol, caveolin-1 exerts, via its binding to NOS3, its inhibitory effect on enzyme activity. The process could constitute one of the mechanisms leading to the decreased NO production associated with heart failure [12,33]. These results emphasize the need to integrate the changes in protein compartmentalisation into the molecular program leading to heart failure.

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