Peroxisome Proliferator-Activated Receptor α Down-Regulation Is Associated With Enhanced Ceramide Levels in Age-Associated Cardiac Hypertrophy

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We used an experimental murine model of accelerated aging, the senescence-accelerated mouse (SAM), to examine the effect of age-associated cardiac hypertrophy on peroxisome proliferator-activated receptor α (PPARα) expression and activity in the heart. Senescence-accelerated prone mice (SAM-P8) showed cardiac hypertrophy compared with senescence-accelerated resistant mice (SAM-R1). Furthermore, a decrease in PPARα messenger RNA (mRNA; 28% reduction, \( p < .001 \)) and protein (47%, \( p < .05 \)) levels and in PPAR DNA-binding activity was observed in SAM-P8 hearts. Increased protein–protein interaction between PPARα and the p65 subunit of nuclear factor-κB (NF-κB) was found, suggesting that this mechanism may prevent PPARα from binding to its response elements. The mRNA levels of PPARα target genes involved in fatty acid use were strongly suppressed in SAM-P8, which was consistent with the accumulation of ceramide in SAM-P8 hearts (2.5-fold induction, \( p < .05 \)). These findings suggest that NF-κB activation in SAM-P8 heart prevents PPARα from binding to its response elements leading to changes in gene expression that may lead to ceramide accumulation in the aged heart.

Advanced age has been identified as a significant risk factor for cardiovascular disease. At the cardiac level, aging is associated with the development of cardiac hypertrophy (1), an independent predictor of cardiovascular mortality (2). There is growing evidence that oxidative stress is the mechanism responsible for the cellular damage associated with aging. Interestingly, the small guanosine triphosphate (GTP)-binding protein Rac1 appears to be involved in the generation of reactive oxygen species (ROS) associated with senescence (3). In addition, overexpression of Rac1 leads to cardiac hypertrophy by activating one of the most important intermediates in this process, nuclear factor-κB (NF-κB), via enhanced degradation of the NF-κB inhibitor IkBα (4,5).

Ceramide accumulation in the heart is associated with cardiac dysfunction and may contribute to the progression from cardiac hypertrophy to heart failure (6–11). Although little is known about the mechanisms responsible for ceramide accumulation during aging, it may stem from changes in the expression of genes related to fatty acid metabolism, which are under the control of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα). In fact, low expression of several genes involved in fatty acid use has been reported in the hearts of PPARα null mice (12), suggesting that this transcription factor is a key regulator of myocardial energetics. To be transcriptionally active, PPARα needs to heterodimerize with the 9-cis retinoic acid receptor (RXR) (NR2B). PPAR-RXR heterodimers bind to DNA-specific sequences called peroxisome proliferator-response elements (PPREs), consisting of an imperfect direct repeat of the consensus binding site for nuclear hormone receptors (AGGTCA) separated by one nucleotide (DR-1). These sequences have been characterized within the promoter regions of PPAR target genes. However, the regulation of gene transcription by PPARα extends beyond its ability to transactivate specific target genes. PPARα is also capable of regulating gene expression independently of binding to DNA through a mechanism termed receptor-dependent transrepression (13). One of these mechanisms involves the physical interaction of PPARα with NF-κB (14).

To determine whether age-associated cardiac hypertrophy causes changes both in the expression or activity of PPARα and in ceramide accumulation, we used the senescence-accelerated mouse (SAM), an experimental model of accelerated aging that has been used extensively to examine the mechanisms responsible for this process. There are two strains: the senescence-accelerated resistant mouse (SAM-R), which ages normally, and the senescence-accelerated prone mouse (SAM-P), which ages at an accelerated rate. The findings presented here show that aging leads to a reduction in PPARα activity through a mechanism that may involve Rac-1–mediated NF-κB activation. As a result of these changes, the expression of PPARα target genes involved in fatty acid use is reduced, leading to ceramide accumulation.
Methods

Animals
SAM-P and SAM-R consist of nine (SAM-P1–3 and 6–11) and three substrains (SAM-R1, 4, and 5), respectively. In this study we used SAM-P8 and SAM-R1 substrains, which were bred under standard conditions with free access to food and water. Male mice were 9 months old at time of use. Animal handling and disposal were performed in accordance with law 5/1995, July 21, of the Generalitat de Catalunya. Mice were killed under pentobarbitone anesthesia, and hearts were excised, weighed, and stored at −80°C.

RNA Preparation and Analysis
Total RNA was isolated by using Ultraspec reagent (Biotex, Houston, TX). Total RNA isolated by this method is undegraded and free of protein contamination. Relative levels of specific messenger RNAs (mRNAs) were assessed by reverse transcription–polymerase chain reaction (RT–PCR) as previously described (15). Complementary DNA (cDNA) was synthesized from RNA samples by mixing 0.5 μg of total RNA, 125 ng of random hexamers as primers in the presence of 50 mM Tris-Cl buffer (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 200 U Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA), 20 U RNAsin (Invitrogen) and 0.5 mM of each dNTP (Sigma, St. Louis, MO) in a total volume of 20 μL. Samples were incubated at 37°C for 60 minutes. A 5-μL aliquot of the RT reaction was then used for subsequent PCR amplification with specific primers.

Each 25-μL PCR contained 5 μL of the RT reaction, 1.2 mM MgCl₂, 200 μM dNTPs, 1.25 μCi [α-32P]dATP (3000 Ci/mmol; Amersham Biosciences, Piscataway, NJ), 1 U Taq polymerase (Invitrogen), 0.5 μg of each primer, and 20 mM Tris-Cl, pH 8.5. To avoid unspecific annealing, cDNA and Taq polymerase were separated from primers and dNTPs by using a layer of paraffin (reaction components were in contact only when the paraffin fused at 60°C). The sequences of the sense and antisense primers used for amplification were as follows: atrial natriuretic factor (Anf), 5′-GGCTGACCTGGAGCATAC-3′ and 5′-GCAAGGTTTTGGGTTGCTTCAT-3′; Pgc-1α, 5′-GGGGCTCTGTCATC-3′; M-Cpt-I, 5′-CTTGGTCA-3′; Mcad, 5′-AGTGAGCAGAC-3′; and adenosyl phosphoribosyl transferase (Aprt), 5′-GGTTCCTTGGGCAATCCCTGA-3′ and 5′-CGGCTCAACACTCCA-3′. PCR was performed in an MJ Research Thermocycler (Waltham, MA) equipped with a pelter system and temperature probe. After initial denaturation for 1 minute at 94°C, PCR was performed for 18 (Ndf), 19 (Mcad, Vlcd), 20 (Anf), 22 (Pgc-1α, Pdk-4), 25 (Pparα, Pparβ, Ppc-1b, Cte, M-Cpt-I, M-Cpt-I and Aprt), 26 (Tnf-α), and 29 (Apo A-I) cycles. Each cycle consisted of denaturation at 92°C for 1 minute, primer annealing at 60°C for 1 minute and 15 seconds, and primer extension at 72°C for 1 minute and 50 seconds. A final 5-minute extension step at 72°C was performed. Five microliters of each PCR sample was separated on a 1-mm-thick 5% polyacrylamide gel. The gels were dried and subjected to autoradiography using Kodak x-ray films to reveal the amplified DNA products. Amplification of each gene yielded a single band of the expected size (Anf: 271 bp, M-Cpt-I: 157 bp, Tnf-α: 284, Pparα: 645 bp, Pparβ: 151 bp, M-Cpt-I: 222 bp, Pdk-4: 167 bp, Cte: 224 bp, Vlcd: 184 bp, Apo A-I: 227 bp, Pgc-1b/β: 185 bp, and Aprt: 339 bp). Preliminary experiments were carried out with various amounts of cDNA to determine nonsaturating conditions of PCR amplification for all the genes studied. Therefore, under these conditions, relative quantification of mRNA was assessed by the RT–PCR method (16). The results for the expression of specific mRNAs are always presented relative to the expression of the control gene (Aprt).

Immunoblotting
Hearts were weighed and homogenized in cold lysis buffer (5 mM Tris-Cl [pH 7.4], 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, aprotinin at 5.4 μg/mL). Cell lysates and nuclear extracts were obtained as previously described (17). Protein concentration was measured by the Bradford method. Proteins (50 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on 10% separation gels and transferred to Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA). Samples were analyzed by Western blot analysis using antibodies that recognize PPARα, Rac1, α65, 1bXβ (Santa Cruz Biotechnology, Santa Cruz, CA), PPARβ/δ (gift of Dr. Walter Wahli, University of Lausanne), and β-tubulin (Sigma). Detection was achieved using the EZ-ECL chemiluminescence detection kit (Biological Industries, Beit Haemek Ltd., Ashrat, Israel). Size of detected proteins was estimated using protein molecular mass standards (Invitrogen Life Technologies).

Electrophoretic Mobility Shift Assays
Electrophoretic mobility shift assays (EMSAs) were performed as previously described (17).
Determination of Ceramide Levels
The content of ceramides in heart was determined by the diacylglycerol kinase method. Briefly, lipids were extracted from approximately 50 mg of heart with 600 μL of chloroform/methanol/1 N HCl (100:100:1, vol/vol/vol). After agitation and centrifugation, the lower phase containing the chloroform-extracted lipids was transferred to a new microfuge tube. Chloroform was evaporated under an N₂ stream. Dried lipids were resuspended in 300 μL of 0.1 N KOH in methanol and incubated for 1 hour at 37°C to eliminate diacylglycerol. Then, 300 μL of phosphate-buffered saline (PBS) was added, and lipid extraction was repeated as indicated above. Lipids were resuspended in 100 μL of reaction buffer (150 μg/100 μL cardiolipin, 280 μM diethylenetriaminepentaacetic acid, 51 mM octyl β-D-glucopyranoside, 50 mM NaCl, 51 mM imidazole, 1 mM EDTA, 12.5 mM MgCl₂, 2 mM dithiothreitol, 0.7% glycerol, 70 μM β-mercaptoethanol, 500 μM ATP, 5 μCi/100 μL [γ-32P]ATP), and 35 ng of diacylglycerol kinase was added to each sample. Reactions were incubated at 30°C for 30 minutes and stopped by the addition of 170 μL of stop buffer (135 mM NaCl, 1.5 mM CaCl₂, 0.5 mM glucose, 10 mM HEPES, pH 7.2) and 30 μL of 100 mM EDTA. Lipids were extracted again with 1 mL of chloroform/methanol/1 N HCl (100:100:1, vol/vol/vol), resuspended in 40 μL of chloroform, spotted onto silica gel thin-layer chromatography (TLC) plates (Whatman Inc., Maidstone, U.K.), and resolved using chloroform/methanol/acetic acid (65:15:5, vol/vol/vol) as a solvent. Spots were measured in a PhosphorImager (Bio-Rad Laboratories, Hercules, CA). Quantification of ceramide mass was obtained by comparison with a standard curve ranging from 0 to 1000 pmol of ceramide-1-phosphate (Sigma), which was processed in parallel with the samples.

Sphingomyelinase Activity
The activity of neutral Mg²⁺-dependent and acid sphingomyelinase was determined as previously reported (18).

Statistical Analyses
Results were obtained from five animals and presented as the mean ± standard deviation (SD). A Student t test was used to determine statistical significance (GraphPad Instat; GraphPad Software Inc., San Diego, CA). Differences were considered significant at p < .05.

RESULTS
SAM-P8 Mice Exhibit Cardiac Hypertrophy
Cardiac hypertrophy is characterized by an increased heart weight/body weight (HW/BW) ratio and the induction of fetal-type genes (e.g., Anf). Therefore, we first examined whether accelerated aging in SAM-P8 led to cardiac hypertrophy. As shown in Figure 1A, the HW/BW ratio was significantly higher (1.4-fold induction, p < .05) in SAM-P8 than in SAM-R1. In addition, mRNA levels for the cardiac hypertrophy marker Anf were 2.2-fold (p < .05) higher in SAM-P8 than in SAM-R1 (Figure 1B). These findings clearly demonstrate that SAM-P8 mice exhibit cardiac hypertrophy.

Rac1 Expression Is Up-Regulated and p65 Translocates to the Nucleus in SAM-P8 Hearts
As Rac1 is involved both in premature senescence (3) and in the development of cardiac hypertrophy (5), we next analyzed the protein levels of this GTP-binding protein. SAM-P8 showed 2.4-fold (p < .01) higher protein levels of Rac1 compared to SAM-R1 (Figure 2A). Furthermore, because development of cardiac hypertrophy by enhanced expression of Rac1 has been associated with NF-κB activation (4), we subsequently evaluated the activation of this transcription factor. NF-κB is a heterodimer, which usually consists of two proteins, the p65 and p50 subunits. In unstimulated cells, NF-κB is found in the cytoplasm bound to IκBα, which prevents it from entering the nucleus. When we examined IκBα protein levels, we found an 82% reduction (p < .001) in its content in SAM-P8 compared to SAM-R1 (Figure 2B). These data suggest enhanced degradation of IκBα, which then releases NF-κB, thereby favoring its translocation to the nucleus. To examine this possibility, we assessed the expression of the p65 subunit in nuclear extracts. As shown in Figure 2C, p65 protein levels were higher (1.4-fold induction, p < .05) in SAM-P8 than in SAM-R1. In agreement with previous reports (4), these data suggest that enhanced Rac1 expression leads to NF-κB activation in the hearts of SAM-P8. To clearly demonstrate
NF-κB activation, we performed EMSA studies. The NF-κB probe formed four main specific complexes with cardiac nuclear proteins (complexes I–IV, Figure 3A), based on competition experiments performed by adding an excess of unlabeled NF-κB oligonucleotide to incubation mixtures. Unexpectedly, NF-κB DNA-binding activity was not significantly different between SAM-R1 and SAM-P8 (Figure 3B). Characterization of NF-κB was performed by incubating nuclear extracts with an antibody directed against the p65 subunit of this transcription factor. Addition of this antibody to incubation mixtures resulted in a supershifted band, thus showing that these complexes contained p65 (Figure 3C). To confirm the lack of enhanced NF-κB binding activity in SAM-P8, we examined the expression of two well-known NF-κB target genes, Mcp-1 and Tnf-α. As shown in Figure 3, D and E, no differences were observed in the levels of these two transcripts between SAM-P8 and SAM-R1. Overall, these data suggest that although p65 translocation from the cytosol to the nucleus occurs in the hearts of SAM-P8 due to increased degradation of IκBα, this is not sufficient to activate NF-κB binding to its response elements. As a result, the expression of its target genes, such as Mcp-1 and Tnf-α, is not modified.

Reduced PPARα Expression and Activity in SAM-P8 Hearts

Although no changes were observed in the expression of NF-κB target genes, increased p65 translocation to the nucleus in SAM-P8 may have affected the activity of PPARs, transcription factors which control the expression of
Figure 3. Lack of changes in the DNA-binding activity of nuclear factor-κB (NF-κB) in senescence-accelerated prone (SAM-P8) hearts. A, Autoradiograph of electrophoretic mobility shift assay (EMSA) performed with a γ-32P-labeled NF-κB nucleotide and cardiac nuclear protein extract (NE) shows four specific complexes (I–IV), based on competition with a molar excess of unlabeled probe. B, Autoradiograph of EMSA performed with a γ-32P-labeled NF-κB nucleotide and NE from senescence-accelerated resistant (SAM-R1) and SAM-P8 mice. C, Supershift analysis performed by incubating NE with an antibody directed against the p65 subunit of NF-κB. Supershifted immune complex (IC) is denoted. All autoradiograph data are representative of three independent experiments. Analysis of the messenger RNA (mRNA) levels of *Mcp-1* (D) and *Tnf-α* (E) in SAM-R1 and SAM-P8 hearts. Representative autoradiogram and quantification normalized to the *Aprt* mRNA levels are shown. Data are expressed as the mean ± standard deviation (SD) of five different animals. *p < .05.
genes involved in fatty acid metabolism (19). In fact, NF-κB activation during cardiac hypertrophy down-regulates PPARα activity, leading to a decrease in the expression of its target genes involved in fatty acid metabolism (20). We therefore evaluated the expression of this transcription factor in SAM-R1 and SAM-P8. PPARα mRNA levels showed a 28% reduction ($p < .001$) in the hearts of SAM-P8 compared to SAM-R1 (Figure 4A). In agreement with the reduction in the transcript levels, a reduction (47%, $p < .05$) was observed in the protein levels of PPARα in SAM-P8 cardiac nuclear extracts (Figure 4B). In contrast, neither the mRNA nor the protein levels of PPARβ/δ were different in the hearts of the two SAM strains studied (Figure 4C and D).

We subsequently performed EMSA to examine the interaction of PPAR with its cis-regulatory element using a 32P-labeled PPRE probe and cardiac nuclear extracts from SAM-R1 and SAM-P8. The PPRE probe formed one main complex with cardiac nuclear proteins (Figure 4E). Competition studies performed with a molar excess of unlabeled probe revealed that this complex represented a specific PPRE–protein interaction. In SAM-P8 cardiac nuclear extracts, a significant reduction was observed in the DNA-binding activity of the complex compared to nuclear extracts from SAM-R1 (Figure 4F). Reduced PPAR DNA-binding activity did not affect the expression of M-Cpt-I and Pdk-4, because the mRNA levels of both genes were not significantly different between SAM-R1 and SAM-P8 (Figure 5A and B), indicating that the basal expression of these genes is maintained by PPARβ/δ, as previously suggested (21). In contrast, when we analyzed the expression of additional PPAR target genes, we observed a reduction in the transcript levels of the cytosolic acyl-CoA thioesterase (Cte) (75%, $p < .05$), which hydrolyses fatty acyl-CoAs to free fatty acids and CoA, and Vlcd, (50%, $p < .05$), involved in mitochondrial oxidation of fatty acids (Figure 5C and D). In addition, we examined the expression of Apo A-I, a gene whose expression is up-regulated in the liver when NF-κB is inactivated through PPARα activation (22). In agreement with the reported activation of NF-κB in SAM-P8, ApoA-I expression was down-regulated (78%, $p < .001$) in this strain strain (Figure 5E).

**Increased p65-PPARα Interaction in SAM-P8 Hearts**

The reduction in PPAR DNA-binding activity in SAM-P8 hearts may result from different molecular mechanisms. First, decreases in PPARα expression may contribute to this reduction. However, because PPARα DNA-binding activity was nearly absent in SAM-P8 hearts, additional mechanisms may also be at work. Second, the reduction in PPAR DNA-binding activity in SAM-P8 hearts may stem from changes in the expression of its coactivators. Interestingly, the expression of two of these coactivators, Pgc-1α and Pgc-1β, is affected by aging in skeletal muscle (23,24). However, the expression of these two coactivators and of two genes regulated by PGC-1α, the medium chain acyl-CoA dehydrogenase (Mcdad) (25) and the subunit 1 of complex I (Nduf) of the electronic transport chain (26), was similar in the two SAM strains, suggesting that this second mechanism is unlikely (Figure 6A–D). Third, the p65 subunit of NF-κB may physically interact with PPARα (14). This association has been described for PPARα and prevents this nuclear receptor from binding to its response element; it thereby inhibits its ability to induce gene transcription (13). Thus, we evaluated whether cardiac hypertrophy in SAM-P8 resulted in enhanced p65-PPARα interaction. Nuclear extracts isolated from hearts were immunoprecipitated using anti-p65 antibody coupled to protein A-agarose beads. Immunoprecipitates were then subjected to SDS–PAGE and immunoblotted with anti-PPARα. Figure 6E demonstrates that, in SAM-P8 hearts, enhanced physical interaction between the p65 subunit of NF-κB and PPARα is evident compared to SAM-R1. This finding suggests that the increased association between these proteins may be a mechanism contributing to the reduced expression of PPAR target genes reported in fatty acid metabolism.

**Enhanced Ceramide Levels in SAM-P8 Hearts**

Finally, we explored the consequences of reduced PPARα activity in SAM-P8 hearts. We have previously reported that the reduced expression of Cte (27) and genes involved in fatty acid mitochondrial oxidation, such as Vlcd, may affect the content of ceramide. Down-regulation of these genes may increase the availability of palmitoyl-CoA, a precursor of sphingolipid synthesis, because the initial step of de novo ceramide synthesis is the formation of 3-ketodihydrophosphoglycine from palmitoyl-CoA and L-serine. To evaluate this possibility, we determined the ceramide content. SAM-P8 exhibited a 2.5-fold ($p < .05$) increase in ceramide content compared with SAM-R1 (Figure 7A). Because ceramide levels can also become elevated by activation of sphingomyelinase, we evaluated neutral and acid sphingomyelinase. No changes were observed in the activity of these enzymes between the two SAM strains (Figure 7B and C), suggesting that the decrease in PPARα activity was responsible for the accumulation of ceramide in hearts of SAM-P8 mice.

**DISCUSSION**

Aging increases the risk of suffering left ventricular hypertrophy (1), a process that when prolonged in the time finally results in congestive heart failure, arrhythmia, and sudden death (28,29). Although aging affects all types of muscle cells, the degree of age-related changes is more pronounced in cardiac myocytes (30). The heart may be especially susceptible to aging due to myocardial dependence on mitochondrial β-oxidation of fatty acids for energy. The expression levels of genes involved in fatty acid uptake, activation, and oxidation in the heart are controlled by the transcription factor PPARα (31). In the present study, we have used an animal model of accelerated senescence to show that age-associated cardiac hypertrophy is associated with myocardial lipid metabolic derangements downstream of the PPARα gene regulatory pathway. Compared with SAM-R1, accelerated senescence in SAM-P8 was accompanied by the presence of cardiac hypertrophy (without preliminary evidences of apoptosis or fibrosis, personal communication, R. Rodríguez-Calvo, M. Vázquez-Carrera, 2006). The development of this process was associated with the activation of the proinflammatory transcription factor NF-κB, which plays a pivotal role in the hypertrophic
Figure 4. Peroxisome proliferator-activated receptor α (PPARα) is down-regulated in senescence-accelerated prone (SAM-P8) hearts. Analysis of the messenger RNA (mRNA) and protein levels of Pparα (A and B) and Pparδ (C and D) in senescence-accelerated resistant (SAM-R1) and SAM-P8 hearts. For the transcript levels, representative autoradiogram and quantification normalized to the Aprt mRNA levels are shown. Data are expressed as the mean ± standard deviation (SD) of five different animals. ***p < .001; *p < .05. Nuclear extracts from SAM-R1 and SAM-P8 hearts were subjected to immunoblot analysis as described in Materials and Methods. Representative immunoblots using PPARα and PPARδ antibodies are shown. Autoradiograph data are representative of three independent experiments. PPAR DNA-binding activity is reduced in nuclear extracts from SAM-P8 hearts. E, Autoradiograph of electrophoretic mobility shift assay (EMSA) performed with a 32P-labeled peroxisome proliferator-response element (PPRE) nucleotide and cardiac nuclear protein extract (NE) shows one (I) specific complex, based on competition with a molar excess of unlabeled probe. F, Autoradiograph of EMSA performed with a 32P-labeled PPRE nucleotide and NE from SAM-R1 and SAM-P8 mice. Autoradiograph data are representative of three independent experiments.
growth of the myocardium (32–34). Activation of NF-κB seems to be initiated in SAM-P8 by enhanced expression of Rac1. The increased expression of this GTP-binding protein in SAM-P8 hearts is in agreement with previous studies showing significant up-regulation of Rac1 in tissues from aged mice when compared with their young counterparts (35). This fact indicates that the SAM-P8 mice offer an effective model of aging for studying the heart. Interestingly, infection of isolated neonatal cardiac myocytes with an adenovirus expressing a constitutively active form of Rac1 leads to cardiac hypertrophy. This occurs via NF-κB activation brought about by enhanced degradation of IκBα (4). Moreover, these findings are consistent with the reduced protein levels of this NF-κB inhibitor as well as the enhanced translocation of p65 to the nucleus observed in this study. However, when we assessed the expression of well-known NF-κB target genes, such as Mcp-1 and Tnf-α, no changes were found between the two SAM strains, suggesting that the higher levels of p65 in the nucleus affect other processes but not the expression of cardiac cytokines. Next, we explored whether PPARα expression or its DNA-binding activity was affected in SAM-P8 hearts. Several studies have reported changes in PPARα expression and activity during the development of cardiac hypertrophy. Thus, PPARα down-regulation has been noted in hypertrophied hearts, both in animal models (36) and in humans (37), whereas PPARα activators have been found to prevent cardiac hypertrophy (38). In the present study, we observed a decrease in Pparα mRNA and protein levels of this transcription factor when comparing SAM-R1 and SAM-P8. In contrast, no changes were observed either in mRNA or in protein Pparδ levels. These findings contrast with other animal models of cardiac hypertrophy, such as pressure overload-induced cardiac hypertrophy, in which we noted a down-regulation in the expression of both Pparα and δ (20). This fact suggests that age-induced cardiac hypertrophy mainly affects PPARα, whereas the expression of other PPAR isoforms present in heart, such as Pparδ, remains unaffected by this process. The reduction in PPARα expression in SAM-P8 hearts may stem from increased production of ROS, because we have previously reported that ROS generation down-regulates Pparα in skeletal muscle cells (39). Consistent with the lack of changes in Pparδ expression in SAM-P8 hearts, no changes were observed in the expression of PPARα and δ-target genes, such as M-Cpt-I and Pdk-4, suggesting that the latter compensates for the reduction of PPARα, as previously reported (21). In contrast to Pdk-4 and M-Cpt-I, the expression of additional PPAR-target genes involved in fatty acid oxidation, such as Cte and Vlcd, was down-regulated in SAM-P8 hearts compared with SAM-R1. Interestingly, the expression of Cte was reduced 75% and that of Vlcd 50% in SAM-P8 hearts compared with SAM-R1, which is similar to the 80% reduction in Cte and the 77% reduction in Vlcd observed in the expression of these genes in hearts from PPARα-null mice (40). Despite these changes, PPARα protein levels were only slightly reduced in SAM-P8 compared with SAM-R1. This lack of correlation suggests that, apart from the reduction in Pparα transcripts, additional mechanisms may be at work in
reducing PPARα DNA-binding activity in SAM-P8 hearts. In fact, enhanced p65-PPARα interaction was observed, suggesting that p65 translocation prevents PPARα binding to its response element. Consistent with the NF-κB-mediated down-regulation of PPARα DNA-binding activity in SAM-P8 hearts, we found a reduction in the transcripts of Apo-AI, which is up-regulated when NF-κB is inactivated via PPARα activation (22). The decreased expression of genes involved in fatty acid oxidation, such as Cte and Vlcod, may lead to the enhanced availability of fatty acids, including palmitoyl-CoA, which is a precursor of de novo ceramide synthesis (41). Because accumulation of these lipid mediators has been associated with cardiac dysfunction (6,11), and since their reduction improves cardiac function (6), we assessed the ceramide content in SAM hearts. We observed the accumulation of ceramide in SAM-P8 hearts compared with those of SAM-R1. This finding suggests that ceramide generation by PPARα down-regulation may be involved in age-associated cardiovascular dysfunctions. Interestingly, transgenic mice with cardiac overexpression of PPARα exhibit increased myocardial fatty acid uptake and ceramide accumulation, cardiac hypertrophy, and left ventricular dilation that is exacerbated by high-fat feeding (8,9). However, prolonged administration of a PPARα agonist does not affect left ventricular dysfunction in a rat model of infarct-induced

Figure 6. Increased peroxisome proliferator-activated receptor α (PPARα) association with the p65 subunit of nuclear factor-κB (NF-κB) in senescence-accelerated prone (SAM-P8) hearts. Analysis of the messenger RNA (mRNA) levels of Pgc-1α (A), Pgc-1β (B), Mcad (C), and Nd1 (D) in senescence-accelerated resistant (SAM-R1) and SAM-P8 hearts. Representative autoradiogram and quantification normalized to the Aprt mRNA levels are shown. Data are expressed as mean ± standard deviation (SD) of five different animals. E, Nuclear extracts (equalized by protein concentrations) from SAM-R1 and SAM-P8 were subjected to immunoprecipitation using anti-p65 antibody coupled to protein A-agarose beads. Immunoprecipitates were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with PPARα antibody. Arrowheads represent the PPARα isoform or the immunoglobulin G signal. The blot data are representative of three separate experiments.
heart failure (42). Therefore, either enhanced or reduced PPARα expression may lead to ceramide accumulation in heart as a result of a mismatch between fatty acid import and utilization.

Overall, although the findings presented here do not provide a cause–effect relationship, they may indicate that age-mediated cardiac hypertrophy is characterized by a decrease in the DNA-binding activity of PPARα, which in turn reduces the expression of its target genes involved in fatty acid oxidation, a process that may be responsible for ceramide accumulation. Reduced PPARα activity correlates with the enhanced protein–protein interaction of this transcription factor with the p65 subunit of NF-κB. These data suggest that those pharmacological strategies geared toward preventing NF-κB activation or toward activating PPARα may prevent the deleterious effects of ceramide accumulation in the aged heart.

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